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ADJUSTING THE SOIL REACTION TO THE CROP

(Editorial)

Potato growers in New Jersey prefer acid soils for their crop. They justify this attitude by the claim that pronounced acidity in the soil assures to them a relative freedom from potato scab. That their claim is essentially sound is borne out not alone by the accumulated experience of many individuals, but also by adequate and convincing experimental study.

The potato growers recognize, at the same time, that a high lime requirement is entirely contrary to the effective and economical maintenance of the soil nitrogen supply. Whenever the lime requirement passes a certain point for any given soil type the growing of clover and alfalfa become increasingly difficult, and the accumulation in the soil of atmospheric nitrogen is accordingly depressed. Hence it becomes necessary to reconcile two opposing tendencies in the growing of large crops of clean tubers. It becomes desirable to create a pronounced acid reaction for the potato crop itself and later to reduce the acidity to a degree that would allow the successful growing of the lime-loving legumes.

A means to this end may be found in powdered sulfur. It would be quite feasible to apply in the early spring, or late in the preceding fall, a sufficient quantity of sulfur to establish any desired degree of acidity for the potato crop. Quantities of from 300 to 1000 pounds per acre of powdered sulfur could thus be employed, the exact amount to be determined by the physical and chemical make-up of each soil. After the potato crop is harvested the existing acidity may be ascertained and corrected by the use of lime. In this manner the soil reaction of any field may be varied from year to year to meet the needs of the potato crop, as well as of the legumes. In a more general way the same method may be employed to maintain such soil-reaction as may seem best, after proper experimental study, for any crop on any soil type.

JACOB G. LIPMAN.

EFFECT OF SULFOFICATION AND NITRIFICATION ON POTASSIUM AND OTHER SOIL CONSTITUENTS

J. W. AMES AND G. E. BOLTZ

Ohio Agricultural Experiment Station

Received for publication February 3, 1919

The experimental work reported in this paper is in part a continuation of investigations pertaining to the effect of sulfur on soils and crops, started by the writers in 1912. In a previous publication (1) we stated that the beneficial effects following the addition of sulfur to soils may result from an increased solubility of soil constituents due to acidity developed from oxidation of sulfur. Some experimental data were also reported at that time, showing that the addition of sulfur to a mixture of rock phosphate and soil increased the acidity of the water extract and the solubility of the phosphate. More recently considerable attention has been given to the influence of sulfofication and nitrification on the availability of rock phosphate.

Microorganisms are considered to be important agencies affecting chemical transformation in soils, but information as to the degree to which the solubilities of soil minerals are changed by organisms is rather limited. The results of different investigations have furnished indications that acids, other than carbonic acid, which are the products of bacterial activities have an appreciable solvent action on calcium. The products of the processes in which various groups of sulfur bacteria, and those producing changes in nitrogen compounds have a part, no doubt react with, and bring about changes in the solubility of other constituents, which may ultimately have an important rôle in crop production.

PROCEDURE

Soil mixtures which included the treatments given in the tables of results were maintained at a temperature of 30°C. for 19 weeks. After the various materials were incorporated with 500-gm. portions of soil, water was added to satisfy 60 per cent of the water-holding capacity, and the mixtures transferred to quart jars which served as containers. Duplicate mixtures of all the treatments were prepared. After the addition of water at regular intervals the contents of the jars were thoroughly mixed.

For the determination of water-soluble constituents, solutions were obtained by extracting 400-gm. portions with 2500 cc. distilled water for 15 hours and filtered through Berkefeld filters under pressure. The soils used in

the work were a silt loam deficient in bases, and a black clay soil. While the calcium carbonate content of the clay is only 300 parts per million, the soil is decidedly basic owing to the presence of basic calcium compounds other than carbonate.

The data reported include determination of potassium, calcium, magnesium, manganese, and aluminum. Indications of the extent to which sulfofication has proceeded are furnished by the quantities of water-soluble sulfur as sulfates. Changes which occurred in the nitrogen content are shown by the amounts of nitric and ammoniacal nitrogen which had accumulated in the soil mixtures at the expiration of the experimental period. Potassium and manganese were determined colorimetrically. Nitric nitrogen was determined by reduction with Devardas alloy and distillation. The usual gravimetric procedures were employed for calcium, magnesium, aluminum and water-soluble sulfur.

Tests were made for iron, but none was present in the solutions excepting a slight trace in two of the mixtures where the acidity was the greatest.

The amounts of the acid and basic ions in the water solutions of the soil mixtures, do not furnish complete evidence of the extent to which reactions may have occurred during the period intervening between the addition of the treatment, and the time the soils were extracted. However, the decided variations found are sufficiently indicative of the effect of the processes on soil constituents. The data for sulfur, acidity, nitrates and ammoniacal nitrogen are given in tables 1 and 2.

ACIDITY OF SOLUTION

A measure of the relative acidity of the solutions was obtained by titration using methyl red as the indicator. The data in tables 1 and 2 show that none of the solutions were acid to methyl red except those from the soils to which sulfur was added, and in these cases it was decidedly decreased where calcium carbonate was present, being practically all neutralized in the case of the silt loam when calcium carbonate was added at the rate of 8000 parts per million of soil.

The amounts of water-soluble ammoniacal nitrogen found where dried blood was present also furnish evidence that the ammonia formed has neutralized a part of the acidity developed by oxidation of sulfur.

All of the solutions of the mixtures from which sulfur was omitted had an alkaline reaction, the alkalinity being somewhat increased by addition of calcium carbonate, and by calcium silicate and blast furnace slag, these three materials supplying approximately equal amounts of calcium. Although the silt loam soil had a lime requirement equivalent to 4000 parts per million of calcium carbonate, the titration of the water extract gives no indication of a deficiency in bases necessary for the maintenance of an alkaline reaction in this soil. Ammonium sulfate has slightly decreased the alkalinity of the

TABLE 1

Silt loam soil

(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	SULFUR	NITRATE NITROGEN	AMMONIACAL NITROGEN	ACIDITY*	ALKALINITY*
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	37	84	13	0	3
2	Calcium carbonate 2 gm.	50	128	0	0	15
3	Dried blood 4 gm.	58	294	67	0	3
4	Dried blood 4 gm., calcium carbonate 2 gm.	95	462	0	0	14
5	Dried blood 4 gm., calcium silicate 2 gm.	122	434	6	0	8
6	Dried blood 4 gm., blast furnace slag 2 gm.	106	478	17	0	4
7	Ammonium sulfate 4 gm.	1,852	54	1,239	0	1
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	1,689	569	178	0	3
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	2,070	100	1,014	0	6
10	Sulfur 2 gm.	2,143	6	50	338	0
11	Sulfur 2 gm., calcium carbonate 4 gm.	1,294	35	27	4	0
12	Sulfur 2 gm., calcium carbonate 2 gm.	1,923	21	46	196	0
13	Sulfur 2 gm., dried blood 4 gm.	1,365	6	246	116	0
14	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	1,559	228	132	10	0
15	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	1,689	29	302	54	0
16	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	1,551	6	279	84	0
17	Sulfur 2 gm., dried blood 4 gm., feldspar 8 gm.	2,053	21	455	166	0
18	Sulfur 2 gm., dried blood 4 gm., feldspar 8 gm. calcium carbonate 4 gm.	1,256	373	172	0	0

*Acidity expressed as H_2SO_4, and *alkalinity as $CaCO_3$.

TABLE 2

Basic clay

(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 400 GM. DRY SOIL	SULFUR	NITRATE NITROGEN	AMMONIACAL NITROGEN	ACIDITY*	ALKALINITY*
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	87	153	3	0	8
2	Dried blood 4 gm.	90	491	25	0	3
3	Ammonium sulfate 4 gm.	1,696	280	737	0	1
4	Sulfur 2 gm.	2,156	16	67	42	0
5	Sulfur 2 gm., dried blood 4 gm.	2,225	12	279	17	0
6	Sulfur 2 gm., ammonium sulfate 4 gm.	3,480	42	1,071	30	0

*Acidity expressed as H_2SO_4, and *alkalinity as $CaCO_3$.

water solutions of both the silt loam soil and the basic clay. The reduction of alkalinity imparted to the solution of the silt loam by calcium carbonate was more pronounced.

The difference in the basicity of the two soils is clearly shown by a comparison of the figures for the acidities of the solutions from the sulfur-treated silt loam, and basic clay soil mixtures. The acidity of the silt loam soil and sulfur mixture is 338. Sulfur added to the basic clay has changed the reaction of the water solution to methyl red from an alkalinity of 8 parts per million to an acidity of 42 parts per million. Practically the same amounts of sulfates, 2143 and 2156 parts per million were found in the solutions from both the silt loam and the basic soil treated with sulfur. This quantity of sulfur is equivalent to 6560 parts per million sulfuric acid, which would require 6690 parts per million of calcium for neutralization. This requirement was largely provided for by calcium and magnesium in the basic clay. A total of 2055 parts per million, calcium and magnesium, was water-soluble in the basic clay, as compared with 473 for the silt loam soil. Calcium and magnesium being deficient in the silt loam, the acidity resulting from oxidation of sulfur has combined with considerable amounts of aluminum and manganese, the sulfates of these elements exceeding the water-soluble calcium and magnesium. Nitrification of dried blood has not caused the water solution of mixtures where nitrates were present to have an acid reaction to methyl red.

The figures obtained for the relative acidity of the solutions do not give an indication of the total acidity produced during the period that sulfur, dried blood, and ammonium sulfate were in contact with soil in the several mixtures. In considering the effects of acidity due to sulfofication or nitrification, it is obvious that the water-soluble sulfates and nitrates will furnish a more correct basis for any deductions made, for the reason that the products of these processes have combined with the soil constituents. The acidity has been neutralized chiefly by calcium carbonate where it was included as a part of the treatment in the silt loam mixtures, and by the natural basic calcium compounds of the black clay.

According to the figures for water-soluble sulfur, approximately 50 per cent of sulfur added at the rate of 4000 parts per million of soil was oxidized where no other additions were made. The effect of calcium carbonate when added in excess of the lime requirement of the silt loam soil, has been to depress the oxidation of sulfur. When half this amount of calcium carbonate was present, the oxidation of sulfur increased, but was less than occurred where no additions other than sulfur were made.

Some of the reactions which occurred between oxidation products of sulfur, and soil constituents in these experimental mixtures will no doubt proceed in field soils, although less actively, and are not dependent on the presence of added sulfur, as sulfur compounds in the soil can be a source of acidity under certain conditions.

Iron sulfide which is a mineral common to many soils is gradually oxidized to the form of sulfate, and then dissolved and hydrolized, with the precipitation of ferric hydrate and the formation of free sulfuric acid.

Sulfur existing in organic combinations may also be changed into a form which can react with soil bases, if the conclusions of Kappen and Quensell (5) concerning the sulfur cycle in soil are correct. They state that hydrogen sulfide produced by action of bacteria, combines with iron-forming iron sulfide which in turn is decomposed, the iron being oxidized and sulfur liberated in a finely divided condition which is oxidized, forming sulfates.

The data for nitric nitrogen show that the formation of nitrates has varied considerably depending on the treatment, nitrification having been stimulated by calcium carbonate, and depressed by the presence of sulfur. Addition of calcium carbonate to the soil deficient in basic calcium compounds has favored the nitrification of its natural supply of nitrogen, as well as that furnished by dried blood and ammonium sulfate.

By comparing the nitric nitrogen results for the three mixtures in which the same amounts of calcium carbonate, calcium silicate and blast furnace slag were included, it will be observed that these three materials have been equally satisfactory for supplying calcium necessary to combine with nitrous acid.

The production of nitric nitrogen from dried blood exceeded that from ammonium sulfate in the basic clay, and in the silt loam soil having no addition of calcium carbonate; the amount formed from ammonium sulfate was less than the accumulation in the untreated soil. The increased base requirement for nitrification of ammonium sulfate as compared with dried blood is evidenced by the nitrate results for both soils. Where calcium carbonate was added to the silt loam at the rate of 4000 parts per million of soil, the formation of nitric nitrogen from dried blood was 462 in contrast with 100 parts per million from ammonium sulfate. When calcium carbonate was supplied at the rate of 8000 parts per million, more nitrates were produced from ammonium sulfate than from dried blood. The natural supply of basic calcium in the clay soil supported a more vigorous nitrification of ammonium sulfate than occurred in the silt loam soil, but the quantity of nitric nitrogen found was less than that from dried blood in this soil.

Acidity resulting from oxidation of sulfur has depressed nitrification. When sulfur and dried blood were in contact, and no calcium carbonate supplied, nitrification was practically inhibited. Where conditions resulting from sulfofication and absence of sufficient base prevented the complete change from proteid to nitric nitrogen, the ammonia formed has partly neutralized acidity.

POTASSIUM SOLUBILITY

The results obtained for water-soluble potassium are of interest because they furnish indications of the effect of salts formed, as the result of acidity developed by micro-organisms, which produce changes in sulfur and nitrogen

carriers. This effect on potassium compounds in the soil, appears to have been a more important factor than the direct action of acidity resulting from the processes of either nitrification or sulfofication.

The solubility of potassium in soil and potassium-bearing minerals as affected by various salts, is a subject to which much attention has been directed. Sodium salts, including nitrate and chloride, liberate potassium according to some investigators, while the opposite conclusion has been arrived at by others. Many conflicting statements also are presented in the literature pertaining to the effect of lime, calcium carbonate, calcium sulfate, and other calcium salts on its solubility. Gardiner and Shorey (4) found that potassium of muscovite and orthoclase was rendered soluble to some extent by treatment with various concentrations of ammonium sulfate solution.

The work of Morse and Curry (9) indicate that lime and gypsum increased the solubility of potassium in feldspar. The experimental work of Bradley (2) with Oregon soils shows that with some soils there was an increase of water-soluble potassium brought about by the addition of gypsum. Investigations reported by McMiller (8) show that various mineral soils mixed with 1 per cent of gypsum, and the water content brought to a point approximating the moisture equivalent and kept in this condition for three months gave marked increases in water-soluble potassium.

Results obtained by Ruprecht and Morse (11) in an investigation of Massachusetts soils which had received additions of ammonium sulfate as a part of the fertilizer treatment, show that there has been a slight solvent action on potassium in the absence of lime.

Lipman and Gericke (6) from experiments conducted for the purpose of determining how calcium carbonate and calcium sulfate affect the solubility of soil constituents, conclude that all soils do not behave alike when treated with these materials. Their results showed an increased solubility in some soils but not in others. The conclusions of Briggs and Breazeale (3) based both on the results of the analyses of the solutions, and on the measurement of the potassium content of wheat seedlings grown in the solutions, are that the availability to plants of the potassium in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum. They found in some instances a marked depression of the solubility in the presence of gypsum.

The results obtained in our experimental work on the effect of nitrification and sulfofication on soil constituents, show that with certain conditions of treatment, there has been some effect on the potassium compounds in the soil, although the amounts of potassium obtained in water solutions are generally much less than the quantities of some of the other elements determined. Considered collectively, the data for potassium, calcium, and sulfur in solution, as well as the ammoniacal nitrogen produced under certain conditions of treatment, indicate that potassium has been liberated chiefly by calcium sulfate and ammonium sulfate.

The data for soluble potassium which are given in tables 3 and 4 show that the process of nitrification, considered from the viewpoint of nitrous acid as a solvent, has affected the solubility less than where the transformation of organic nitrogen occurred in the presence of oxidized sulfur. Whatever effect the change in the nitrogen furnished by the treatment with dried blood has had, may be due to calcium or other nitrates formed, rather than to the

TABLE 3

Effect of nitrification on potassium in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	NITRATE NITROGEN
		p.p.m.	*p.p.m.*
1	None	18	84
2	Calcium carbonate 2 gm.	7	128
3	Dried blood 4 gm.	30	294
4	Dried blood 4 gm., calcium carbonate 2 gm.	24	462
5	Dried blood 4 gm., calcium silicate 2 gm.	33	434
6	Dried blood 4 gm., blast furnace slag 2 gm.	39	478
7	Ammonium sulfate 4 gm.	30	54
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	21	569
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	26	100

TABLE 4

Effect of nitrification and sulfofication on potassium in basic clay soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	NITRATE NITROGEN	SULFUR
		p.p.m.	*p.p.m.*	*p.p.m.*
1	None	31	153	87
2	Dried blood 4 gm.	44	491	90
3	Ammonium sulfate 4 gm.	72	280	1,696
4	Sulfur 2 gm.	48	16	2,156
5	Sulfur 2 gm., dried blood 4 gm.	60	12	2,225
6	Sulfur 2 gm., ammonium sulfate 4 gm.	85	42	3,480

direct action of acidity. In the acid and basic soils used in this investigation, the addition of dried blood has caused a small increase of water-soluble potassium.

It will be noted from the results in table 5, showing the effect of sulfofication on potassium in the acid silt loam, that where dried blood and sulfur were the additions made, a further increased solvent action has taken place. This is considered to be the result of the action of ammonium sulfate, since in the presence of sulfur gradually being oxidized, the ammoniacal nitrogen formed has combined with the sulfur acidity. The quantity of ammoniacal nitrogen found at the end of the experimental period was 246 parts per million.

The amount actually present, however, was in excess of this, for ammonia added to soil cannot all be recovered except by distillation with magnesium oxide. The addition of sulfur and dried blood to the basic soil has affected the solubility of potassium in a similar manner. The potassium obtained in solution from the acid silt loam mixtures which included dried blood and sulfur are given in table 5, and the results for the mixtures to which either dried blood or ammonium sulfate were added are shown in table 3

In the acid silt loam the addition of ammonium sulfate has not affected the solubility of potassium to the same extent as the transformations of sulfur and dried blood when added together, which are considered to have formed ammonium sulfate. The potassium solubility of the basic clay mixtures which are presented in table 4 show that added ammonium sulfate has had an appreciable effect; the increases in this soil being greater than those found in the water solution from the acid soil where ammonium sulfate was added.

TABLE 5

Effect of sulfofication on potassium in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	POTASSIUM	SULFUR	ACIDITY*
		p.p.m.	*p.p.m.*	*p.p.m.*
1	Sulfur 2 gm.	18	2,143	338
2	Sulfur 2 gm., calcium carbonate 4 gm.	32	1,294	4
3	Sulfur 2 gm., calcium carbonate 2 gm.	53	1,923	196
4	Sulfur 2 gm., dried blood 4 gm.	52	1,365	116
5	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	43	1,559	10
6	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	49	1,689	54
7	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	62	1,551	84

*Acidity expressed as H_2SO_4.

Considering the effect of oxidation of sulfur on potassium, the results show that oxidized sulfur has produced less solubility in the acid soil than in the basic clay. The effect of sulfur treatment alone, and when added with blood and calcium carbonate, is shown by the results for the acid soil in table 5 and for the basic clay in table 4. It will be noted that in the acid soil, there has been no increase of water-soluble potassium where sulfur was the only addition, but when included with calcium carbonate or dried blood the solubility of the potassium was found to be appreciably increased.

Sulfofication proceeded no less actively in the absence of calcium carbonate and dried blood, in fact the quantity of sulfur from the acid soil mixture treated with sulfur alone was greater than when these materials were added. The acidity of the water extract was also greater when dried blood and calcium carbonate were not added.

The formation of ammonium sulfate has been offered as the explanation of the effect of sulfur when used with dried blood. With calcium carbonate

added, the acidity from the oxidation of sulfur has reacted with calcium carbonate and a considerable amount of calcium sulfate has been formed in the mixtures. This is evident from the amounts of water-soluble calcium obtained from the silt loam mixtures receiving additions of calcium carbonate and sulfur, as well as in the basic clay where no calcium carbonate was supplied. The amounts of water-soluble calcium found in the various mixtures, including those treated with sulfur are presented with the data in tables 7 and 8.

The presence of calcium sulfate in the mixtures which have just been discussed is considered to have been the agency chiefly responsible for the liberation of potassium from the insoluble combinations in which it existed in these soils.

TABLE 6

Solubility of potassium supplied by feldspar in sand and soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. SAND AND SOIL	POTASSIUM	NITRATE NITROGEN	AMMONIACAL NITROGEN	SULFUR
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	Sand	0	0	0	0
2	Sand, sulfur 2 gm.	0	0	0	42
3	Sand, sulfur 2 gm., calcium carbonate 2 gm.	0	0	0	503
4	Sand, feldspar 8 gm.	4	0	0	0
5	Sand, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm.	52	27	325	478
6	Sand, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm., calcium carbonate 2 gm.	33	21	58	1,937
7	Soil	18	84	13	37
8	Soil, dried blood 4 gm., sulfur 2 gm.	52	6	245	1,365
9	Soil, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm.	96	21	465	2,053
10	Soil, feldspar 8 gm., dried blood 4 gm., sulfur 2 gm., calcium carbonate 2 gm.	58	373	172	1,256

The general conception of the action of calcium sulfate in liberating potassium, has been that this phenomenon is brought about through the exchange of calcium with other soil bases. A more recent hypothesis has been advanced by Nolte (10) who considers that calcium sulfate acts on the soil by means of both its constituents, double decomposition occurring with the mineral compounds of the soil.

Where the soils were treated with sulfur, dried blood and calcium carbonate in the case of the acid silt loam, there was no doubt a combined effect of ammonium sulfate and calcium sulfate. Calcium nitrate produced where nitrification of dried blood occurred, especially in the presence of calcium carbonate, calcium silicate and blast furnace slag, may have had an effect on potassium compounds in the soil.

Since soluble potassium salts added to soils are very rapidly fixed to such an extent that only small quantities can be recovered by extraction with water, it is very probable that the amounts of potassium obtained do not

represent the total quantities which may have been changed from insoluble compounds in the soil, to more soluble forms, by the processes taking place in the soil mixtures during the period of incubation.

Further indications of the effect produced by nitrogen and sulfur transformations are furnished by data obtained where feldspar was included with sand and with soil. The treatment and the amounts of water-soluble potassium obtained are stated in table 6. Dried blood and sulfur where 8 gm. of feldspar was added to 500-gm. portions of sand has decidedly increased the solubility of potassium of the feldspar. With calcium carbonate included in the treatment, solubility is decreased, and a similar effect has taken place where feldspar was included with dried blood and sulfur in an acid silt loam soil mixture.

SOLUBLE CALCIUM AND MAGNESIUM

It is apparent from the results for calcium in tables 7 and 8 that considerable amounts, present as combinations other than carbonate, have been changed to more soluble compounds in the differently treated soil mixtures. Where dried blood was nitrified in the silt loam soil, which is deficient in basic calcium, the calcium in solution was 255 parts per million as compared with 111 parts per million from the untreated soil. The addition of calcium carbonate stimulated nitrification, consequently a larger amount of calcium nitrate was formed. Calcium supplied as silicate, and by blast furnace slag was made soluble in a similar manner.

The calcium in solution from the basic clay soil was dissolved from its natural supply, since no calcium carbonate was added to this soil which contains a large amount of readily salifiable base, most of which is evidently combined as silicate, and a small proportion as carbonate. The total calcium content of this soil is 9400 parts per million as compared with 2300 in the other soil. Nitrification of dried blood, and the original nitrogen of the clay soil, proceeded to the extent that the soluble calcium was considerably in excess of the amounts extracted from similar mixtures of the silt loam. Lyon and Bizzell (7) have reported data which show that a large removal of nitrates from an unplanted soil, where conditions were favorable for nitrification, was associated with a correspondingly large loss of calcium.

Ammonium sulfate, independent of the ammonia being nitrified, reacted with calcium either originally present or added as carbonate, and the solutions of the soil mixtures in which this treatment was included contained large amounts of calcium. These results for soluble calcium, corroborate the information obtained from the analyses of drainage water from soils which have received application of ammonium sulfate, that this material depletes the supply of basic calcium compounds.

Acidity resulting from the oxidation of sulfur has freely attacked the soil constituents, including calcium, the amount in solution varying with the

TABLE 7

Data for calcium, magnesium, aluminum and manganese in silt loam soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	CALCIUM	MAGNESIUM	ALUMINUM	MANGANESE	SULFUR	NITRATE NITROGEN
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	111	30	0	0	37	84
2	Calcium carbonate 2 gm.	270	13	0	0	50	128
3	Dried blood 4 gm.	255	54	0	34	58	294
4	Dried blood 4 gm., calcium carbonate 2 gm.	736	34	0	0	95	462
5	Dried blood 4 gm., calcium silicate 2 gm.	549	57	0	0	122	434
6	Dried blood, 4 gm., blast furnace slag 2 gm.	596	50	0	0	106	478
7	Ammonium sulfate 4 gm.	355	80	0	38	1,852	54
8	Ammonium sulfate 4 gm., calcium carbonate 4 gm.	2,199	87	0	69	1,689	569
9	Ammonium sulfate 4 gm., calcium carbonate 2 gm.	1,067	85	0	18	2,070	100
10	Sulfur 2 gm.	370	103	660	468	2,143	6
11	Sulfur 2 gm., calcium carbonate 4 gm.	858	108	68	297	1,294	35
12	Sulfur 2 gm., calcium carbonate 2 gm.	785	64	413	471	1,923	21
13	Sulfur 2 gm., dried blood 4 gm.	317	76	234	330	1,365	6
14	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 4 gm.	1,505	113	11	325	1,559	228
15	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 2 gm.	858	111	103	419	1,689	29
16	Sulfur 2 gm., dried blood 4 gm., calcium carbonate 1 gm.	417	92	185	395	1,551	6

TABLE 8

Data for calcium, magnesium, aluminum and manganese in basic clay soil
(Expressed as parts per million of dry soil mixtures)

NO.	ADDITIONS TO 500 GM. DRY SOIL	CALCIUM	MAGNESIUM	ALUMINUM	MANGANESE	SULFUR	NITRATE NITROGEN
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	None	245	41	0	0	87	153
2	Dried blood 4 gm.	668	97	0	16	90	491
3	Ammonium sulfate 4 gm.	1,297	183	0	8	1,696	280
4	Sulfur 2 gm.	1,773	282	40	48	2,156	16
5	Sulfur 2 gm., dried blood 4 gm.	1,763	248	52	44	2,225	12
6	Sulfur 2 gm., ammonium sulfate 4 gm.	2,080	275	79	56	3,480	42

treatment. Where sulfur and dried blood were in contact, calcium was changed to a soluble form by a combination of reactions which resulted in the formation of ammonium sulfate. In these mixtures the basicity was not sufficient to neutralize the acidity produced, so that complete nitrification of

ammonia was prevented, and a part of the ammonia combined with sulfuric acid resulting from oxidation of sulfur.

Comparatively small amounts of magnesium were changed to a soluble state considering the total content of the soils. The silt loam soil contains approximately 2800 parts per million, slightly more than the total calcium; and the magnesium in the basic clay is 7000 parts per million. Where sulfur was not added to the silt loam, the soluble magnesium exceeds the aluminum and manganese found, but is decidedly less than the calcium brought into solution. In the solutions from mixtures where considerable acidity was produced by the oxidation of sulfur, some small increases are observed but they are not comparable with the much larger amounts of soluble manganese and aluminum.

Larger quantities of soluble magnesium were found in the solutions from the basic clay, which is in accord with the total magnesium of the soil. The concentration of magnesium in solutions for the clay soil was also greater than that of manganese and aluminum being the reverse of what was found for the silt loam soil.

ALUMINUM, IRON, AND MANGANESE

So far as evidence is furnished by the quantities of aluminum in the several solutions, the process of nitrification has had no solvent action on aluminum compounds in the two soils used in this experimental work.

No aluminum was extracted from mixtures which did not include sulfur as part of the treatment. The largest amount of aluminum sulfate was present in solution from soil where sulfur was oxidized in the absence of calcium carbonate.

The addition of calcium carbonate at the rate of 8000 parts per million of soil, decreased the solubility of aluminum to 68 parts per million, as compared with 660 parts per million, where basic calcium and magnesium compounds were sufficient to enter into combination with only a small part of the sulfuric acid.

Acidity resulting from the oxidation of sulfur in the basic clay reacted chiefly with calcium and magnesium. Much smaller amounts of manganese and aluminum sulfates were formed in this soil than in the silt loam.

Nitrification of dried blood had a slight effect on manganese in both soils. Ammonium sulfate also had a slight solvent action. Manganese, existing in the soil as oxides and decomposed silicates was freely attacked by the acidity resulting from oxidation of sulfur. The manganese found in the solution generally exceeds the aluminum. No indication was obtained that iron compounds were appreciably affected by the processes of nitrification and sulfofication.

SUMMARY

The indications obtained from this experimental work are as follows:

Nitrification of dried blood and oxidation of sulfur in soil mixtures increased the water-soluble potassium.

The liberation of potassium was brought about by salts formed, rather than by the direct action of acidity on insoluble potassium compounds. Calcium nitrate was present where nitrification was stimulated by calcium carbonate. Ammonium sulfate was formed, by the reaction between sulfuric acid and ammonia from dried blood, where a deficiency of basic calcium compounds restricted the nitrification of ammonia. Considerable amounts of calcium, aluminum and manganese sulfate were also present in certain mixtures.

Ammonium sulfate, independent of the ammonia being nitrified, affected the solubility of potassium.

Calcium naturally present in the soil as silicate, and in other combinations has been readily attacked by the acidity resulting from nitrification and sulfofication. Ammonium sulfate was also an active solvent of calcium.

Magnesium compounds were much more resistant to the action of these solvent agencies.

Large quantities of aluminum and manganese were converted into soluble forms where sulfur was oxidized in an acid silt loam soil. Small amounts of these elements were made soluble by this action in a basic clay. Nitrification had no effect on aluminum.

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NOTES ON RECENT WORK CONCERNING ACID SOILS

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In 1916 Gillespie (2) and the authors (8) showed by direct hydrogen electrode or colorimetric measurements that many soils are acid. This term signifies that the solutions of such soils have a greater concentration of H-ions than OH-ions. Subsequent work in other laboratories as well as in our own has substantiated this fact and has made clear the importance of recognizing and applying these simple principles of chemistry. Recently two articles have appeared in SOIL SCIENCE dealing with this subject on which the following brief comment is thought necessary, since it appears desirable to make every effort to bring about agreement on the chemical principles underlying soil problems.

Gillespie (3) has quoted from a previous article by the authors the expression "The rational treatment of acid soils requires that sufficient lime be added to bring the soil to a neutral or slightly alkaline reaction." This sentence when considered apart from the context of the several articles on acid soils gives an erroneous impression of our views. By the above expression, perhaps unhappily chosen, we have meant only to indicate that if one proceeded on the assumption, implied in so many of the so-called lime-requirement methods, that a neutral or slightly alkaline reaction is desirable for plant growth, then the most appropriate method would be to add lime to the soil in the quantities necessary to produce the desired reaction as measured by the hydrogen electrode. We did not intend to express any opinion with regard to the optimal reaction for the growth of plants, as evidenced by the fact that one of us (4, 5) has since investigated this point and has not found that an H-ion concentration comparable to that of most acid soils is inhibitive to the growth of many common plants of agricultural interest. Consequently, we have not been and are not now opposed to Gillespie's view that no arbitrary P_H value can be assigned as optimal for plant growth.

We are also in agreement with Gillespie in his statement that the attainment of a neutral reaction in the soil does not mean that all the water-soluble acids are neutralized. Our own experiments have in fact indicated that lime may be added to an acid soil until a slightly alkaline reaction is maintained for a number of days or weeks and that subsequently after a longer period of time an acid reaction reappears (though of a less concentration of H-ion than at the beginning). Theoretically it might be possible to add a sufficient quan-

tity of base to neutralize all acid substances present, but limitations of time, fineness of division of the soil, etc., would perhaps make such a neutralization impracticable. Finally, we desire to say that except to correct the impression noted above, our general viewpoint and method of attack are in entire accord with those of Dr. Gillespie.

The second article to which we wish to refer has been published recently by Rice and Osugi (7) in SOIL SCIENCE. This article deals with the inversion of cane sugar when heated in contact with soils. The data presented are very interesting but certain conclusions require a critical examination. In the first place, it is claimed in the course of their discussions that the determinations of H-ion concentration as carried out by Gillespie and by the authors may be affected by the carbon dioxide present and so lead to erroneous conclusions with respect to the nature of soil acidity. In reply to this we should like to

TABLE 1

Effect of boiling soil suspensions on PH *value*

SOIL NUMBER	SOIL SUSPENSION WITHOUT PREVIOUS HEATING	SOIL SUSPENSION AFTER BOILING*
	P_H	P_H
1	5.5	5.5
2	4.4	4.1
3	5.3	5.3
4	5.7	5.5
5	7.3	7.2

* The suspensions were boiled for ½ hour and then cooled while passing a rapid stream of hydrogen gas. The values given represent the average of several closely agreeing determinations.

call attention to one of our previous articles (6) in which specific data appear on this point. We have proved that even percentages of carbon dioxide as high as 9 per cent did not appreciably affect the reaction of acid soils. That the results would not be significantly changed by previous boiling of the suspension is evident from the determinations given in table 1.

In the experiments of Rice and Osugi it was found that many soils gave a very marked inversion of cane sugar while their extracts displayed scarcely any inverting power. Moreover the P_H values of the sugar extracts (determined by Sörensen's method) were in nearly every case practically neutral. Since the H-ion is considered to be the chief catalytic agency involved in the inversion of cane sugar, Rice and Osugi conclude that the so-called "true acidity" of soils is determinable only through the catalytic method and is generally the property of the insoluble portion of the soil. If the generally accepted belief be true that the catalysis of the inversion of sugar is a function of the H-ion concentration,[1] then it is obvious that the rate of inversion is first of all

[1] Acree, among others, has presented evidence supporting the view that non-ionized acids may also take part in the inversion of cane sugar. Acree, S. F., and Layng, T. E., 1913, Reaction of both ions and non-ionized forms of electrolytes. *In* Amer. Chem. Jour. v. 49, p. 345–368.)

dependent upon the solution, which is necessarily the only phase of such a system carrying H-ions. The H-ions, however, must be in equilibrium with the undissolved soil mass, although we do not understand the exact nature of this equilibrium. It is difficult to comprehend how the solid portion of the soil can effect inversion independently of the solution with which it is in contact. It is true that the solid phase exerts a considerable influence on the rate of inversion, but this must be attributed to modifications and readjustments of the solution effective for inversion as will be referred to later.

Since some of the explanations advanced by Rice and Osugi are at variance with our conceptions of these phenomena we conducted a number of experiments on the inverting power of soils and their extracts. The method used

TABLE 2

PH *values and inverting power of soils and soil extracts*

SOIL NO.	SOIL SUSPENSION	WATER EXTRACT OF SOIL*	WATER EXTRACT OF SOIL AFTER BOILING	SUGAR EXTRACT OF SOIL	SUGAR INVERTED BY WATER EXTRACTS OF SOILS: CALCIUM AS DEXTROSE PER 50 CC.	SUGAR INVERTED IN PRESENCE OF SOIL: CALCIUM AS DEXTROSE PER 50 CC.
	P_H	P_H	P_H	P_H	*gm.*	*gm.*
†15	3.4				0.163	0.268
12	4.5			4.2		0.147
6	4.4	4.4	4.6	4.4	0.050	0.114
2	4.3	4.4	5.0	3.8	0.069	0.122
5	4.6			4.8	0.030	0.071
13	5.6	5.3	5.5	5.1		0.026
14	5.9	5.1	5.6	5.6		0.025
10	5.4	5.1	5.1	5.7		0.010
11	6.3	5.7	5.5	6.2		0.004
7	7.5			6.8	0.003	0.014
8	7.5			6.5	0.001	0.009
9	7.5			6.8	0.001	0.002

* In a number of cases the water extract of the soil was too cloudy to make possible a satisfactory estimation of the H-ion concentration by the colorimetric method.

† Sample of silicic acid.

was the same as that proposed by Rice and Osugi; namely heating the 10 per cent sugar solution and the soil or extract for one hour at a temperature of 85°C. and then determining the amount of copper reduced. The results are summarized in table 2.

The first point to be noted is that the soils of highest H-ion concentration caused the greatest inversion and the very slightly acid soils and alkaline soils gave only a slight inversion. A very marked inversion was also produced by the extracts of the soils of H-ion concentration. It is unquestionably true that a larger inversion resulted when the sugar solution was heated in the presence of the solid soil particles. The data available at present do not enable us to explain satisfactorily why the presence of the solid particles should induce a more rapid rate of inversion than the solution by itself. Possibly a greater

concentration of H-ion is present in the films of solution closely associated with the soil particles, or it may be that H-ions are temporarily combined with carbohydrate molecules during the inversion and that the presence of the solid phase maintains a more constant concentration. It is to be remembered that the absolute quantity of H-ion in solution at any one time is very small and that only a small fraction of the total sugar is inverted. The above explanations are, of course, only conjectures which at present cannot be substantiated by direct evidence. But whatever may be the exact function of the solid material, it is fair to conclude from our data that large inversion occurs only in soils of distinctly acid reaction, with the greatest inversion occurring coincidently with the highest H-ion concentration of the soil suspension as well as of the water and the sugar extracts. The inversion of sugar and the subsequent reduction of copper are very complex processes in which other factors than the concentration of H-ion may be important, so that when only slight quantities are inverted these are not necessarily to be correlated with the exact H-ion concentration.

The data of the above table also gave direct evidence that acid soils do give acid filtrates. The extracts were filtered through no. 590 S and S filter paper, and the H-ion concentration determined by the indicator method of Clark and Lubs (1). The filtrates gave acid reactions, generally of a magnitude very similar to those obtained with the suspensions. Boiling the extract for several minutes produced no large changes in H-ion concentration. Gillespie has previously shown that the colorimetric and hydrogen electrode determinations are usually in much closer agreement than the above when all suitable precautions are taken. It is difficult to understand why the statement is so often made that acid soils give alkaline extracts. If the latter are made with pure water and if the filtering medium is entirely inert, such a change of reaction can not occur. As pointed out in a previous paper (6) the reactions of boiled extracts made with water saturated with carbon dioxide have no bearing on the H-ion concentrations of the naturally existing soil solutions.

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THE EFFECT OF CERTAIN COLLOIDAL SUBSTANCES ON THE GROWTH OF WHEAT SEEDLINGS[1]

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The power of a soil to retain water against the force of gravity, to counteract the bad odors of decaying organic matter, and to modify the concentration of a solution with which it is in contact, is due wholly or in part to the phenomenon of absorption. Since the chemical composition of the soil is so complex it is difficult to ascertain to what extent the process of absorption from solution by the soil in a given case is chemical and to what extent it is physical. The absorption by some of the relatively insoluble soil constituents such as silica, and iron and aluminum oxides is probably physical and is termed adsorption. Within the last few years considerable work has been done pertaining to the absorption of salts and ions from solution by soils and other finely divided materials. Little attention, however, has been given to the effect this might have upon plant growth.

As a rule, adsorption is positive, that is, the concentration of a salt in the bulk of a solution is usually reduced by introducing absorbing surfaces into the solution. Consequently, the concentration of the salt at the interface is greater than that of the bulk. The object of this work was to attempt to answer the following question: Is the change in concentration due to solid adsorbing surfaces sufficient to modify the production of dry matter in a plant?

REVIEW OF LITERATURE

Gregoire (5) grew barley to maturity in Detimer's nutrient solution to which was added three-tenths of a per cent of silica in one treatment, and the same per cent of aluminum oxide in another treatment. Solution cultures were used as checks. There was a decided increase in the yield of dry matter in the silica cultures, and an increase in the case of the aluminum oxide cultures, but less than for the silica. More than 43.5 per cent of the ash of the plants from the silica culture consisted of silica, while less than 5 per cent of silica was present in the ash of the check. This author believes that the increase was due largely to the absorption of silica by the plants and its consequent utilization in growth. The ash of the plant in the alumi-

[1] A thesis submitted to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

num oxide culture contained 6 per cent alumina, while there was less than 1 per cent alumina in the ash from the check.

By growing wheat plants in solutions of several different densities, Lyon and Bizzell (8) showed that for the nutrient solution used the dry matter produced per unit of transpiration increased with the density. An increase in this ratio is considered indicative of greater density of the nutrient solution. They found that plants grown in crushed quartz had a lower transpiration ratio than when grown in nutrient solution of the same concentration. It appeared, therefore, that the actual densities in the region of the absorbing surface of the roots may be greater in the case of quartz. Plants grown in crushed quartz develop an abundance of root hairs and the root hairs come in intimate contact with quartz grains. It is possible, therefore, that the root hairs are in the absorbed layer, and are, therefore, feeding from a denser solution.

McCall (9) grew wheat plants in Shive's 3-salt solution when a solid medium representing somewhat the condition of the soil, but without the biological complications was present. He used a granite-ware pot provided with a pipe at the bottom for drainage. The solution was renewed every third day. The dry weights of the plants grown in the quartz sand were compared with those obtained by Shive. Sand cultures gave a greater dry weight. It is suggested by the author that the thickness of the adsorbed layer is less than the outer cell wall covering the adsorbing protoplasm, and since the absorbing protoplasm does not come in direct contact with this concentrated layer, the salts here are unavailable except in so far as the slow process of diffusion takes place.

It is also suggested in this article that selective adsorption may be an important factor in modifying plant growth. Thus the author calls attention to the fact that a better growth was produced in the sand than in the water cultures as the ratio of calcium nitrate to magnesium sulfate increased. This is explained by assuming that the NO_3 radicle produces a beneficial effect, that the magnesium sulfate antagonizes this effect, and that the magnesium sulfate more than the NO_3 would be adsorbed by the sand, and, therefore, less magnesium sulfate would be present in a condition to antagonize the favorable influence of the nitrate radicle. The results giving rise to this suggestion are subject to criticism since there is no record of the treatment having been duplicated.

Gile and Carrero (3) grew rice plants in acid, neutral and alkaline nutrient solutions when supplied with 0.002 gm. and 0.008 gm. of iron per liter from the following sources of iron: ferrous sulfate, ferric chloride, dialyzed iron, ferric citrate, ferric tartrate. The dialyzed iron was prepared by the ordinary method. It was entirely inadequate for the plants, for they were strongly chlorotic at all times.

Negeli (10), Dandeno (2), True and Oglevee (12, 13), Breazeale (1), Livingston (7), Jensen (6), and others have shown that the introduction of

finely divided substances like carbon black into nutrient solutions causes increased growth. This effect has been attributed to the action of the solid on certain organic and inorganic toxins.

True and Oglevee (13) advanced the theory that the adsorbing substances increase the growth rate by reducing the concentration of the toxic material, acting in the same way as the addition of water would by leaving a fewer number of ions or molecules in the free solution.

According to this theory it would be possible to introduce a solid substance into a nutrient solution and reduce the actual concentration of the solution in the region of the absorbing surface. If the theory is correct, this application is true, it being only necessary to select the proper adsorbent.

Dandeno (2) suggests that the increase in growth may be due to a decrease in the diffusion rate, while Jensen (6) adds a third possibility: viz., chemical change induced by the presence of the finely divided material.

The literature fails to give conclusive evidence as to whether the introduction of a solid adsorbent into a nutrient solution increases or decreases the density of that solution at the absorbing surface of the roots. The present work was intended as a contribution to this subject.

PLAN OF THE INVESTIGATION

Baker's analyzed salts were used in making up the nutrient solution. The salts were weighed and dissolved in distilled water, giving a stock solution for each salt. The following table shows the concentration and composition of each stock solution:

Calcium nitrate	216 gm. in 2000 cc. of water
Potassium chloride	60 gm. in 2000 cc. of water
Magnesium sulfate	48 gm. in 2000 cc. of water
Ferric sulfate	4 gm. in 1000 cc. of water
Mono potassium phosphate	60 gm. in 1000 cc. of water

A complete nutrient solution containing about 4500 parts per million was made up by adding 25 cc. of each solution to distilled water, as follows: The calcium nitrate and potassium chloride solutions were added to 250 cc. of distilled water. The ferric sulfate, magnesium sulfate and potassium phosphate solutions were diluted each with 175 cc. of distilled water and put in 3 different containers. The diluted ferric sulfate and magnesium sulfate solutions were mixed and then added to the potassium chloride and calcium nitrate mixture. Finally, the potassium phosphate solution was added and the mixture shaken. At the latter addition there was always a slight precipitation. The solution was not filtered but was always shaken thoroughly before using.

Galgalos wheat was used. The method of germinating and growing the wheat up to the point of placing it under treatment was the same in all cases and was as follows: A few hundred clean seeds were placed in a wide-mouth bottle provided with a cork with one hole through which a piece of glass

tubing was inserted, one end reaching to the bottom of the bottle. The other end was connected with the water tap. By providing a cork too large or slightly too small, the water would run over the top without allowing the seeds to pass out. The seeds were always kept in running water for 48 hours. They were then transferred to a large floating disc and here kept in running water until four or five centimeters in height. This required about four days. The floating disc was a piece of stiff wire netting covered with paraffin. Many small holes were made in the paraffin to furnish a contact between the seedlings and water. Many more plants were grown to this stage than were required for the experiment, which made more easy a selection of plants of uniform top and root growth. Strong healthy plants were always selected for the cultures. The endosperms were carefully removed and 6 plants threaded into holes in a cork that would fit the container to be used. The threading was most easily done by carefully pressing the tops together and passing them into the hole, and then working the seedling into the desired position. Each cork with the 6 plants was then placed in the container filled with tap water. It appeared early in the work that the placing of the plants under treatment as soon as the endosperm was removed was not satisfactory, for many of the plants died under these conditions, while if kept in tap water for a short time they developed very well. Four small sticks placed in holes bored near the circumference of the cork and tied together by twine furnished support for the tops of the growing plant.

Wherever possible the distilled water which was to be used in culture work was treated either with carbon black or with precipitated oxide of iron or aluminum. This treatment of the distilled water consisted in adding 15 gm. of the material to a liter of water, shaking and allowing to stand for a few hours and filtering.

The hydroxides of iron and aluminum were prepared from the chlorides of these elements, by precipitation from solution by NH_4OH and then washing free of ammonium chloride. The iron hydroxide was used at the rate of 10 gm. of dry material calculated as Fe_2O_3 and the aluminum hydroxide at the rate of 8 gm. Al_2O_3 per liter of distilled water. They were kept moist and used in the same way as the carbon black. In the following discussion the term "treated" nutrient solution has reference to the distilled water used.

During the period that plants were growing under treatment, they were kept in the greenhouse at a temperature of about 55°F. during the night and 60°F. during the day.

At harvest the tops were removed and dried at a temperature of 90°C. In order to obviate lack of uniformity resulting from an unequal distribution of heat in the greenhouse, the relative positions of the cultures were changed at each weighing. The cultures were weighed or the solutions were renewed twice each week.

The following materials, all of which have been found by different investigators to adsorb from solution, were used in the present work: agar, silica, crushed quartz, and hydroxides of iron and aluminum.

Agar cultures

The agar solution was prepared by adding to the dry agar sufficient distilled water to make a 2 per cent solution and boiling the mixture in a double boiler for 2 hours. The resulting solution was then cooled to a temperature of 60°–75°C., and the proper amount of standard nutrient solution and distilled water added to give the desired dilution of nutrient salts. In the earlier part of the work a one per cent solution of agar was used, but difficulty was experienced in getting the tender roots of the wheat plants to penetrate this medium. It was found more satisfactory to use one-half per cent agar as the plant roots would easily penetrate this medium. After the agar medium had cooled to laboratory temperature the plants which had previously been selected and threaded into the corks were placed in the agar. Water cultures of the same concentration of nutrient salts were prepared as checks, the plants being placed in the water and agar cultures at approximately the same time. In certain of the cultures the solutions were not renewed but each culture was weighed twice each week and water added equivalent to the loss in weight.

In other cases the solutions were renewed by lifting the cork carrying the seedlings, discarding the old medium, rinsing the container, and adding 300 cc. fresh medium from stock solutions of the proper strength. The results of these experiments are given in tables 1, 2 and 3.

The results with agar were not entirely satisfactory, but the figures present some interesting indications. Referring to table 1, it will be seen that in concentrations of 500 p.p.m. of nutrient salts, agar was beneficial, while with a concentration of 2000 p.p.m. the agar was harmful. A similar effect may be noticed in table 2, where in the 500 p.p.m. solution, agar was beneficial while in the 1000 p.p.m. solution, agar was harmful. With the exception of the 85 p.p.m. concentration, more dilute solutions were benefited by agar, while the higher concentrations were injured. It is conceivable that with the more concentrated solutions the agar rendered the effective concentration at the surface of the particles so great as to become toxic to plants.

TABLE 1

Dry weights of wheat plants grown in solution and in agar cultures. Solution not renewed; volume of solution 930 cc.; each result the average of 4 cultures

TREATMENT	CONCENTRATION	PERIOD OF GROWTH	DRY WEIGHT			DIFFERENCE	
			Tops	Roots	Whole plant		
	p.p.m.	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Nutrient solution + 1 per cent of agar	500	30	0.3240	0.1262	0.4502	+0.1740	62.9
Nutrient solution	500	30	0.1799	0.0963	0.2762		
Nutrient solution + 1 per cent of agar	2000	37	0.3011	0.1133	0.4144	−0.1098	−24.0
Nutrient solution	2000	37	0.3568	0.1674	0.5242		

TABLE 2

Dry weights of wheat plants grown in a nutrient solution and in a nutrient solution with 1 per cent of agar. Volume of solution 500 cc.; solution not renewed; each result the average of 4 cultures

TREATMENT	CONCENTRATION	PERIOD OF GROWTH	WEIGHT OF			DIFFERENCE	
			Tops	Roots	Whole plant		
	p.p.m.	*days*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Distilled water		18	0.0918	0.0311	0.1229		
Distilled water + agar		18	0.1239	0.0524	0.1763	+0.0534	+42.6
Nutrient solution	85	20	0.1643	0.0488	0.2252		
Nutrient solution + agar	85	20	0.1494	0.0478	0.1972	−0.0280	−12.4
Nutrient solution	250	29	0.2367	0.0604	0.2971		
Nutrient solution + agar	250	29	0.2790	0.0667	0.3457	+0.0486	+16.3
Nutrient solution	500	36	0.2721	0.0569	0.3290		
Nutrient solution + agar	500	36	0.3217	0.0681	0.3898	+0.0608	+18.1
Nutrient solution	1000	36	0.4420	0.1076	0.5496		
Nutrient solution + agar	1000	36	0.3075	0.0636	0.3711	−0.1786	−32.4

+ Indicates increase in agar culture compared with check.

− Indicates decrease in agar culture compared with check.

TABLE 3

Dry weights of wheat plants when grown for 5 weeks in a nutrient solution and in a nutrient solution to which was added 0.5 per cent agar. Solution renewed twice per week; volume of solution 300 cc.; concentration 750 p.p.m.

TREATMENT	LAB. NO.	WEIGHT OF		
		Tops	Roots	Whole plant
		gm.	*gm.*	*gm.*
Nutrient solution	80	0.4042	0.0905	0.4947
	81	0.5175	0.1133	0.6308
	82	0.4355	0.0858	0.5213
	83	0.6204	0.1401	0.7605
	84	0.5456	0.1198	0.6654
	85	0.5479	0.1479	0.6958
	86	0.4456	0.0839	0.5295
	87	0.5531	0.1179	0.6710
	88	0.5372	0.1239	0.6611
	89	0.4485	0.0923	0.5408
Average		0.5056	0.1114	0.6171
Nutrient solution + 0.5 per cent of agar	90	0.3951	0.0884	0.4845
	91	0.3786	0.0677	0.4363
	92	0.5104	0.0921	0.6025
	93	0.3970	0.0896	0.4866
	94	0.4119	0.0945	0.5064
	95	0.3629	0.0882	0.4511
	96	0.4551	0.0816	0.5373
	97	0.7340	0.1569	0.8909
	98	0.6323	0.1440	0.7763
	99	0.5562	0.0927	0.6489
Average		0.4829	0.0995	0.5824

Difference between the solution and agar cultures, −0.0347 gm. or − 5.62 per cent.

Silica cultures

Colloidal silica was prepared by dialysis following in general the method used by Graham (4). A preliminary test was made as follows:

With a pipette approximately 10 cc. of a 10 per cent solution of hydrochloric acid were measured into an Erlenmeyer flask. Sodium silicate (a 10 per cent solution) was slowly added from a burette while the mixture was shaken vigorously. As the sodium silicate increased, the mixture gradually became more viscous and finally set to a gel. The amount of silicate solutions required to form the gel was then noted. In preparing a quantity of the mixture for dialysis, only one-half to two-thirds of the total required sodium silicate solution was used. In a mixture of these proportions the silica will not set until the chlorides are reduced to a very small percentage.

Two kinds of dialyzers were used. A very efficient dialyzer was made by taking 5 feet of a heavy quality of parchment paper tubing, tying one end carefully and then telescoping it into a piece of glass tubing of a little less length but of somewhat greater diameter. The end of the glass tube containing the closed end of the parchment paper tubing was provided with a one-hole stopper and a short piece of glass tubing sealed into the hole in this stopper. One end of a rubber tube was then attached to the small glass tubing, the other end leading to a container of distilled water which furnished a head of three or more feet. The parchment paper was always soaked for two or three days in distilled water, and carefully tested for leaks. The solution to be dialyzed was run into the parchment paper tubing from the open end which was then tied and crowded into the glass tube. This end of the dialyzer, which was supported about two feet above the opposite end, was provided with a one-hole stopper and glass tubing for drainage to the sink. A constant stream of distilled water was then forced from the lower to the upper end, flowing outside of the parchment tubing and carrying the chlorides which diffused through the paper into the drainage. Such an arrangement exposes a large surface to a steady stream of water and therefore makes a very efficient dialyzer. As we were unable to obtain a sufficient amount of the parchment paper tubing for all the work with silica, a modified Stern dialyzer was also used. This consists of two pans each of about five liters capacity arranged so that a stream of distilled water comes in at the bottom of one, fills the pan and overflows evenly at the top. The other pan has parchment paper drawn tightly over the top and tied, and a hole made in the bottom large enough to insert a funnel. This pan is placed upon the first pan bottom up so that the parchment paper comes in contact with the water in the first pan. Dialysis begins at once when the mixture of sodium silicate and hydrochloric acid is put in the top pan. A few short pieces of filter paper laid between the two pans help to establish a uniform distribution of the water as it flows from the pan. About five liters could be dialyzed at one time with this apparatus which requires a period of nearly three weeks

with the water running continuously. As dialysis continues, the viscosity of the sol gradually increases, finally setting to a gel. The extent to which the silica can be purified before setting depends, of course, upon the concentration of the silica. Thus in one case with 2.8 per cent of silica, gelation began when nearly 5000 p.p.m. of chlorides were present, while in another case with 1.5 per cent of silica, gelation did not begin until the chloride content was reduced to 700 p.p.m. The silica was never allowed to set firmly in the dialyzer, but was siphoned into a large container and then enough measured into the culture jars to give 1 per cent of silica when diluted to 500 cc. The dilutions were made from the standard nutrient solution or from this solution and distilled water. Before measuring the colloidal silica solution into the jars for the cultures, total solids and chlorides were determined and the latter calculated as sodium chloride. As it was not practicable to remove all chlorides from the silica, sodium chloride was added to the cultures containing no silica so that all culture media contained the same amount of

TABLE 4

Dry weights of wheat seedlings grown for 27 days in pure nutrient solutions and in nutrient solutions containing 1 per cent silica

TREATMENT	DRY WEIGHT OF TOPS	INCREASE DUE TO SILICA	
	gm.	*gm.*	*per cent*
Nutrient solution 85 p.p.m.	0.3627		
Nutrient solution 85 p.p.m. + silica 1 per cent.	0.4276	0.0649	17.8
Nutrient solution 250 p.p.m.	0.4594		
Nutrient solution 250 p.p.m. + silica 1 per cent.	0.5936	0.1342	29.2

sodium chloride. Wheat plants were selected, threaded into corks and transferred from tap water to the culture solutions in the manner described for agar cultures. Within two to three days the plants growing in nutrient solutions containing 1 per cent of silica were larger and had better color than those growing in pure nutrient solutions. This difference was maintained until harvest. The results of this experiment are given in table 4.

The weights of roots are not given in the above table since it was not always possible to make an accurate separation from the adhering gel.

The figures in table 4 show that the silica was decidedly beneficial to the wheat seedlings. It occurred to the author that the effect might be due to increased absorption of silica by the plant. Determinations of silica in the seedlings showed that the suspicion was well founded. The data may be seen by reference to table 5. An attempt was made to obviate this disturbing factor by introducing into one set of cultures 50 p.p.m. of colloidal silica, on the assumption that this amount would supply the requirements of the plant for silicon. Accordingly, three sets of cultures were set up in the manner already described. Four concentrations of nutrient salts were used. The data are given in table 5.

TABLE 5

Dry weights and silica content of wheat seedlings grown in nutrient solutions and in nutrient solutions containing silica

TREATMENT	DRY WEIGHT OF TOPS	SILICA IN DRY MATTER
	gm.	*per cent*
Nutrient solution 85 p.p.m.	0.2628	5.3
Nutrient solution 85 p.p.m. + colloidal silica 50 p.p.m.	0.2875	8.3
Nutrient solution 85 p.p.m. + silica gel 1 per cent.	0.4669	16.0
Nutrient 250 p.p.m.	0.5635	3.1
Nutrient solution 250 p.p.m. + colloidal silica 50 p.p.m.	0.5846	5.1
Nutrient solution 250 p.p.m. + silica gel 1 per cent.	1.0540	20.2
Nutrient solution 500 p.p.m.	0.5743	2.8
Nutrient solution 500 p.p.m. + colloidal silica 50 p.p.m.	0.6389	3.9
Nutrient solution 500 p.p.m. + silica gel 1 per cent.	1.1404	22.8
Nutrient solution 1000 p.p.m.	0.9688	2.0
Nutrient solution 1000 p.p.m. + colloidal silica 50 p.p.m.	0.7961	3.6
Nutrient solution 1000 p.p.m. + silica gel 1 per cent.	1.4235	31.3

The results show a striking correlation between silica in the culture medium, growth of plant, and silica in plant. The medium does not, therefore, fulfill the requirement of an inert colloidal substance and is, therefore, not suitable for the problem undertaken in this study. The data are given merely for the benefit of those who may wish to undertake similar work.

Sand cultures

The amount of salt adsorbed from a solution by an adsorbent depends upon the surface exposed by the adsorpent. If a change in the concentration of the nutrient salts due to adsorption is sufficient to modify the production of dry matter in plants, the difference ought to be increased when the fineness of the adsorbent is increased. Quartz sand was selected as the adsorbent on the assumption that being very insoluble it would not supply nutrients to the plant. Three different grades of sand were secured from the New England Quartz Company of New York City. These are known commercially as no. 000, 1 and 2. A mechanical analysis is given in table 6.

The sands were purified by heating with hydrochloric acid (1: 3) for about ten hours, washing with distilled water until free from chlorides, thoroughly drying and storing for future use.

In growing plants either in soil or in water cultures it is necessary to replace the water lost by transpiration and evaporation. With water cultures this renewal is accomplished either by adding at frequent intervals sufficient distilled water to maintain the original volume or by discarding the old medium entirely and adding fresh nutrient solution equivalent to the original amount of the old. The second procedure is preferable since it eliminates the possible accumulation of toxic excreta. With quartz sand cultures renewal of solutions has not been practiced. The method used by McCall

in which solutions were renewed but old sand retained did not take into account the possible saturated condition of the quartz after continual use. The writer has devised a method for renewing not only the nutrient solutions but also the quartz.

For this purpose glass fruit jars of 1 pint capacity were used. A hole $\frac{1}{2}$ inch in diameter was drilled in the bottom of each jar. The holes were stoppered and plants which had been selected and threaded into the corks were transferred to the jars which were to be used in the experiment. A volume of 250 or 300 cc. of nutrient solution of the desired concentration was transferred to the jar by means of a funnel, a large hole having previously been made in the cork for this purpose. Seven hundred grams of quartz sand was then weighed, a large funnel inserted in the hole of the stopper, and the sand allowed to fall gently into the solution. By using a small scoop and

TABLE 6

Mechanical analysis of quartz sands

	NUMBER 2	NUMBER 1	NUMBER 000
	per cent	*per cent*	*per cent*
Fine gravel (2–1 mm.)	0.08	0	1.53
Coarse sand (1.0–0.5 mm.)	21.09	0	2.63
Medium sand (0.5–0.25 mm.)	68.93	2.25	8.93
Fine sand (0.25–0.1 mm.)	8.45	92.51	69.62
Very fine sand (0.1–0.05 mm.)	0.16	1.91	12.27
Silt and clay (less than 0.05 mm.)	0.86	3.29	4.78
	99.57	99.96	99.76

sprinkling the sand into the funnel the stream of sand falling into the solution could easily be regulated. The roots of the plants were completely buried in the sand. At renewal periods the old sand and solution were removed by first taking out the small stopper at the bottom of the container, and then allowing a gentle stream of distilled water to run in at the top, the cork holding the plants remaining in place. Being under a low head this water never ran swiftly. A large glass siphon was used, and to give flexibility to that end which was inserted through the hole in the stopper containing the plants, a piece of rubber tubing was attached. The fresh solution and sand were added in the same manner as the first addition.

All solutions were renewed twice per week. The checks were solution cultures and were grown in the same amount and same concentration of nutrient solution as the sand cultures. The distilled water used in all cultures was treated with carbon black. The process of renewal just described appears not to be detrimental to the seedlings, as the comparison in table 7 will show.

TABLE 7

Dry weights of wheat seedlings grown in quartz to which nutrient solutions were added

	WEIGHTS OF SEEDLINGS GROWN IN	
	Quartz sand number 2	Quartz sand number 000
	gm.	*gm.*
Nutrient solution and sand renewed twice each week.....	0.6551	0.8033
Nutrient solution and sand not renewed, but distilled water added.....	0.5089	0.5888

* These figures indicate a better development where solutions were renewed.

In tables 8 and 9 are given the results of experiments showing the effect of quartz sands of different degrees of fineness on the growth of wheat.

TABLE 8

Dry weights of wheat plants grown 35 days in nutrient solutions with and without quartz sand of different degrees of fineness.

(Concentration of nutrient solution 750 p.p.m.; sand and nutrient solution changed twice per week.)

TREATMENT	DRY WEIGHT OF WHEAT SEEDLINGS, TOPS ONLY						AVERAGE
	1	2	3	4	5	6	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nutrient solution.....	0.2624	0.2717	0.2011	0.2816	0.2449	0.2339	0.2492
Nutrient solution + sand no. 2.....	0.2158	0.2440	0.2362	0.2448	0.1920	0.2663	0.2332
Nutrient solution + sand no. 1.....	0.2382	0.2245	0.2279	0.2280	0.2368	0.2312	0.2311
Nutrient solution + sand no. 000.....	0.1911	0.2298	0.2179	0.2356	0.1984	0.1842	0.2073

TABLE 9

Dry weights of wheat plants grown for 28 days in nutrient solutions with and without qnartz sand of different degrees of fineness.

(Concentration of nutrient solution 370 p.p.m.; sand and nutrient solution renewed twice each week.)

TREATMENT	DRY WEIGHT OF WHEAT SEEDLINGS, TOPS ONLY					AVERAGE
	1	2	3	4	5	
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Nutrient solution.....	1.0442	1.1595	1.0149	1.2198	1.1670	1.1211
Nutrient solution + sand no. 2..	0.6827	0.6175	0.6871	0.6696	0.6038	0.6551
Nutrient solution + sand no. 000.	0.8749	0.8826	0.8930	0.6370	0.8743	0.8033

It will be seen that the amount of dry matter produced is greater for a nutrient solution of a given concentration than for the same concentration in sand cultures. Although the differences are small, it seems sufficient to indicate that the decrease in concentration of the nutrient solution by the sand may be a factor causing a smaller production of dry matter.

Ferric hydroxide cultures

It occurred to the author that hydroxides of iron and aluminum might be suitable for a study of adsorption. These substances are relatively insoluble, are colloidal in character, and supposedly would not furnish nutrients to increase plant growth. The ferric hydroxide was prepared as follows: A 10 per cent solution of chemically pure ferric chloride was prepared and placed in a large bottle. Ammonium hydroxide was then added carefully until the mixture was faintly alkaline to litmus. The bottle was then filled with distilled water, stoppered, shaken thoroughly, and allowed to stand until the precipitate had settled. The supernatant liquid, was siphoned off and the washings repeated until free from chlorides. After the last washing the suspension was allowed to settle for several weeks and the supernatant liquid drawn off. A sample of the remaining suspension was used for determination of the ferric hydroxide and the remainder used in the cultures to be described. The cultures were made up so that all solutions contained the same concentration when calculated on a basis of total volume of water and total nutrient salts. Sufficient ferric hydroxide suspension was used to give 1.5 per cent ferric hydroxide calculated on volume of culture solution. The plants were grown in glass tumblers of 300 cc. capacity, the plants being previously selected and threaded into corks and placed in the different cultures. In all cases the entire medium was renewed twice each week. The results of the experiment are given in table 10.

No difference could be seen in the general appearance of the ferric hydroxide cultures and the checks during the early part of the growing period, except that the latter in most cases developed a heavier growth. Towards the end of the growing period, however, the plants in the ferric hydroxide cultures had a somewhat darker green color. The duration of the growing period was in every case determined by the plants in the iron, there being a more marked yellowing of the ends of the leaves in these cultures. These effects may result from an excessive use of iron in the metabolism of the plant, although, in view of the work of Gile and Carrero and from the insolubility of the colloidal iron, this would not seem to be the case.

In every case the dry weight of the tops is less for the plants grown in ferric hydroxide cultures.

The colloidal ferric hydroxide adheres to the roots forming a very tenacious covering. The roots were always washed carefully in distilled water before drying, but it was impossible to remove all of the iron oxide from them.

TABLE 10

Dry weights of wheat plants grown in nutrient solution and in nutrient solution containing ferric hydroxide calculated as Fe_2O_3

TREATMENT	WEIGHT OF TOPS ONLY	AVERAGE
Length of growing period—5 weeks		
	gm.	*gm.*
Nutrient solution, 750 p.p.m.	0.4042	
	0.5175	
	0.4355	
	0.6204	
	0.5456	
	0.5479	
	0.4456	
	0.5531	
	0.5372	
	0.4485	0.5056
Nutrient solution 750 p.p.m. + ferric hydroxide 1.5 per cent	0.3706	
	0.3283	
	0.4044	
	0.5868	
	0.4408	
	0.3499	
	0.3507	
	0.3457	
	0.4374	
	0.3876	0.4002
Length of growing period—30 days		
Nutrient solution 750 p.p.m.	1.2430	
	1.7361	
	1.9641	
	1.6378	
	1.6540	1.6490
Nutrient solution 750 p.p.m. + ferric hydroxide 1.5 per cent	0.8435	
	1.1832	
	0.7775	
	0.7405	
	0.9629	0.9015
Length of growing period—31 days		
Nutrient solution 370 p.p.m.	1.1147	
	1.3210	
	1.6747	
	1.5611	
	1.5614	
	1.4955	1.4894
Nutrient solution 370 p.p.m. + ferric hydroxide 1.5 per cent	0.9688	
	0.9892	
	1.1689	
	1.0984	
	1.0029	
	1.0927	1.0535

Aluminum hydroxide cultures

The procedure already described for the preparation of ferric hydroxide was followed in preparing the aluminum hydroxide, the chloride of aluminum being used in the place of ferric chloride. The suspension was made of such strength that the cultures contained 2 per cent of aluminum hydroxide calculated as Al_2O_3. The experiment was performed at the same time and under the same conditions as were the ferric hydroxide cultures. The results are given in table 11.

TABLE 11

Dry weights of wheat plants grown in nutrient solution and in a nutrient solution containing 2 per cent of aluminum hydroxide calculated as Al_2O_3

TREATMENT	WEIGHT OF TOPS ONLY	AVERAGE
Length of growing period—31 days		
	gm.	*gm.*
Nutrient solution 370 p.p.m.	1.1147	
	1.3210	
	1.6747	
	1.5611	
	1.5614	
	1.4955	1.4894
Nutrient solution 370 p.p.m. + aluminum hydroxide 2 per cent	1.1188	
	1.4504	
	1.2624	
	1.1612	1.2481
Nutrient solution 750 p.p.m.	1.6490	1.6490
Nutrient solution 750 p.p.m. treatment with Al_2O_3	1.3607	
	1.4984	
	1.3131	
	1.5164	1.4222
Nutrient solution 750 p.p.m. + aluminum hydroxide 2 per cent	1.4411	
	1.6344	
	1.1921	
	1.7367	
	1.5414	1.5091

Difference between nutrient solution (treated) and nutrient solution with Al_2O_3 gm. — 0.0530, per cent — 2.8.

The results in table 11 show that aluminum hydroxide is similar to ferric hydroxide in that its addition to nutrient solutions caused a decrease in the growth of the wheat plants.

SUMMARY

The effect of adding agar to nutrient solutions was to increase the growth of wheat seedlings in low concentrations and to decrease the growth in higher concentrations of nutrient salts. The introduction of colloidal silica into nutrient solutions resulted in increased weight of wheat seedlings. The increase was apparently due to direct absorption of silica by the plant and not to a change in the effective concentration of the nutrient solution. Silica gel is, therefore, considered unsuited for studies of the character described in this paper. The introduction of quartzs and, ferric hydroxide, and aluminum hydroxide into nutrient solutions resulted in decreased growth of wheat seedlings. It appears that these substances by their absorptive properties reduce the effective concentration of the nutrient solution.

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THE LONGEVITY OF B. RADICICOLA ON LEGUME SEEDS

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As a practical problem, the longevity of the nitrogen-gathering bactera upon the coats of legume seeds, is an important one. Since inoculation of legumes is so widely practiced, and of the several methods in vogue, probably the seed inoculation method is the one most generally used, it is desirable to know the best inoculant to use as well as the most efficient and economical way of applying it.

Oftentimes the farmer inoculates his seed, and on account of rain, or for other reasons, he cannot plant it at once. The question immediately arises—How long can enough bacteria survive on these seeds to produce good inoculation of the plants? Obviously this time varies with climatic conditions, methods of applying the bacteria to the seeds and the variety of legume. Other factors may also cause bacteria to perish soon after inoculation.

The idea was conceived that if the bacteria survived on the seedcoats of the common legumes for a sufficient length of time, then the seed dealers could attend to the inoculation of seed and thus save the planter the time and trouble, besides carrying it out in a more uniform and economical manner. Most large dealers in seeds are also dealers in legume cultures and for this reason would be able to do the work cheaply. Many of the failures in alfalfa growing may be attributed to ignorance of the necessity of inoculation. If all seed could be inoculated before planting, better results in the growing of certain legumes for the first time would be obtained. Many farmers have an antipathy against handling bacteria, a term which implies and is often considered synomonous with disease. If legume seeds could come ready to plant, it would also further their introduction into certain localities and thus be of great economic gain to the country.

Very few experiments have been carried out on this question, and these are not exhaustive enough to draw any practical deductions from. Temple (7), of the Georgia Agricultural Experiment Station, has shown that *Bacillus radicicola* may live for months in dry soii and on the seedcoats of cowpeas. It is a well known fact that both the symbiotic, as well as the non-symbiotic, nitrogen-fixing bacteria may live for years in dry soil. The writer found that dry soil, taken from a soybean field 18 months before, caused copious nodule production on soybean plants, which were grown in uninoculated soil. Plants grown in check pots, side by side, showed no inoculation. Thus it

has been already established to a certain extent that the nodule organisms can, under certain conditions, withstand considerable desiccation.

The cotton cultures, of the U. S. Department of Agriculture (5) failed, because the bacteria soon died on the dried strands of cotton. Chester (1) in 1907 described some experiments showing extremely variable results regarding the desiccation of root tubercle bacteria. His work consisted in the main of depositing pure cultures of *B. radicicola* on cotton gauze or wool, drying at 35°C. (a temperature far above that to which this organism is commonly exposed and at which it nearly always fails to grow on agar plates), and testing for viability at fixed periods after the drying was complete. He found that the cotton became sterile at periods varying from a few hours to 62 hours in one case. He states, "*Ps. radicicola* has little power to withstand drying and its preservation in the dry state on cotton is inadvisable". This organism also died quickly when deposited upon glass in thin films. As a result of a short experiment with alfalfa seed, he states, "*Ps. radicicola* when dried on seed perish very quickly".

Noyes and Cromer (6) made tests to determine the amount of infected soil or commercial cultures necessary to produce inoculation. The main criticism with this work is that the amounts used per acre were much too small. The writer (3, 4) showed that commerical cultures for legume inoculation vary greatly in quality. Of all the cultures tested, the muck and soil cultures gave the best results. He also showed that certain seeds, like the soybean, were very difficult to inoculate. Several of the cultures tested contained sufficient bacteria to give 100 cells per seed under ordinary conditions of planting. It is very desirable to know just how long these cells will remain alive and active on the seedcoat of the legume before the seed is planted or after it has been planted in dry soil. For this reason, the following series of experiments have been carried out.

EXPERIMENTAL

It was first necessary to determine a satisfactory method of sterilizing the seeds, in order to insure reliable results from the experiments. Several well known substances were tried in this work and a comparison test made. Soybean seed, of the Black Eyebrow variety, was used in all of the following tests.

A. Formalin

First the seeds were soaked in a 1 per cent formalin (40 per cent formaldehyde), solution for 15 minutes, rinsed in five washings of sterile water and placed aseptically in tubes of beef broth, one seed to the tube. After 48 hours but 20 per cent of the tubes showed no growth. The germination of the seed was reduced from 86.5 to 56.0 per cent. Solutions of formalin, varying in strength from 1 to 5 per cent, were next tried in the same manner, except that the time of immersion of the seeds was reduced to 3 minutes. In

no case was sterilization complete, although in the case of the 4 per cent and 5 per cent solutions 60 and 80 per cent, respectively, of the tubes were sterile. In the second series, solutions of formalin ranging from 1 to 5 per cent were allowed to act upon the seeds for 10 minutes. Here again germination was seriously interfered with. It was found that seeds treated with a 4 or 5 per cent formalin solution for 10 minutes were usually free from bacteria, but still retained viable fungus spores.

B. Hydrogen peroxide

Acting upon a suggestion of Dr. John W. Shive, plant physiologist of the New Jersey Agricultural Experiment Station, hydrogen peroxide was used to treat the seeds. They were soaked in a 2 per cent solution for 5 minutes. Only a small part of the seeds were found to be sterile, but instead of injuring germination, it was greatly enhanced and the early growth of the seeds was much stimulated. This may have been due to the action of the liberated oxygen upon the seeds. The method was not tried further.

C. Calcium hypochloride

Ordinary bleach showing on analysis 33 per cent available chlorine was used in such dilution as to give a solution containing approximately 6 per cent chlorine. The seeds were immersed for periods of 5 and 10 minutes. This chemical gave good results and did not materially affect germination. From 80 to 100 per cent of the tubes containing treated seeds were found sterile after 48 hours.

D. Mercuric bichloride

The seeds were placed in a 1 to 500 solution of mercuric bichloride for 5 minutes, removed aseptically and washed in 5 changes of sterile distilled water. The whole time occupied about 10 minutes. Upon testing these seeds in bouillon tubes, practically all (95 per cent) were sterile. Germination was somewhat reduced, but this was not a vital factor in this experiment, as twice as many seeds were planted as were allowed to grow to maturity. The qualitative analysis of 5 of the treated seeds showed only the merest trace of mercury. It is not believed that this would exert a toxic effect upon the bacteria to be added later to the seeds.

Treatment with bichloride of mercury gave such satisfactory results that it was finally adopted in the work. Probably bleach is just as good, as Wilson (8) has shown, but the writer began the use of the mercury salt and continued it throughout the experiments.

MEDIA USED IN THE WORK

A comparison of several media was made, the results being reported elsewhere (3). A modified Ashby's medium was found to give the best all round results besides being easily made up. This medium contains mannite, 10

gm., mono-potassium phosphate 0.2 gm., magnesium sulfate 0.2 gm., calcium sulfate 0.1 gm., calcium carbonate 1.0 gm. These salts were dissolved in 1,000 cc. of distilled water, to which 12 gm. of agar had been added. The agar was purified previous to use by soaking in water for 6 hours to remove soluble substances. The mono-potassium phosphate was neutralized separately with normal potassium hydrate. While testing commerical cultures of legume bacteria sometime previous to carrying on this experiment, a medium was used containing 15 gm. of agar and 12 gm. of mannite per liter, in addition to the above salts. It is believed, however, that the smaller amounts of mannite and agar are preferable, for on comparing, the media colonies of *B. radicicola* were larger and more characteristic. This is in accord with previous observations of the writer (2).

Soybean seed of the Black Eyebrow variety were used in the main experiment, although some seeds of the Early Brown variety were used in the preliminary experiment. These seeds had been previously treated with bichloride of mercury and sterile water and had been dried between sterile blotting pads in a quiet, dark room. The inoculum was prepared by crushing 25 gm. of fresh, thoroughly washed soybean nodules in 500 cc. of sterile water. This infusion was then filtered through several layers of cheese cloth. The seeds were placed in this filtered nodule infusion for the following periods of time: 2 minutes, 20 minutes, 6 hours and 24 hours. After this they were removed, and placed on sterile blotting paper in a quiet, dry, dark room for several hours. On becoming dry, the seeds were transferred aseptically to sterile paper bags and stored away. The first seeds were tested bacteriologically, as well as being planted in pots, one hour after soaking in nodule infusion. They were also tested after 6 hours, 24 hours, 72 hours, 10 days, 30 days, 90 days, 6 months and 9 months.

The bacteriological technique used was as follows: 5 seeds (5 seeds weigh about 1 gm.) were taken from the bag with sterile forceps, placed in Erlenmeyer flasks containing 100 cc. of sterile tap water, allowed to stand 20 minutes, so as to thoroughly soften the seed to insure adhering cells being easily washed off, agitated 5 minutes in an electric soil shaker, and plated on modified Ashby's agar. The dilutions commonly used were: 1:1,000; 1:100,000; and 1:1,000,000. Plates were always poured in duplicate, hence 6 plates were required for each test. These were incubated at 25° C. for 10 days, after which they were counted.

For the pot tests, where seeds were tested for their ability to produce nodules, small earthenware pots, containing 2 pounds of soil were used. The pots were sterilized in an autoclave, at 10 pounds pressure, for 20 minutes. The soil used was a Sassafras sandy loam, taken from a remote part of the college farm, which had been shown repeatedly not to contain *B. radicicola* of the soybean or alfalfa variety. The soil was made up to optimum moisture content. Seven seeds were planted in each pot, 5 being allowed to grow for the period of the experiment. Duplicate pots were always planted to help

avoid possible errors. Distilled water was used at first to moisten the soil, but it was later found that the greenhouse tap water contained no *B. radicicola*, hence tap water was afterwards used. After 30 days the pots were removed to another building, the soil and plants removed, and the nodules carefully counted. The results of the bacteriological tests and the vegetation experiments obtained in the preliminary experiment are given in table 1.

TABLE 1

Results of preliminary experiments with Early Brown soybeans

LENGTH OF TIME AFTER INOCULATION THAT TEST WAS MADE		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT				
		2 minutes	20 minutes	1 hour	6 hours	24 hours
1 hour..	Bacteria per seed*	1,300,000	1,600,000	5,400,000	6,300,000	10,000,000
	Nodules per plant†	5.8	7.1	7.2	3.9	14.7
6 hours...	Bacteria per seed	720,000	1,300,000	4,200,000	900,000	7,300,000
	Nodules per plant	6.1	7.0	8.1	9.9	14.0
24 hours...	Bacteria per seed	360,000	620,000	3,100,000	3,900,000	5,300,000
	Nodules per plant	11.0	3.1	6.7	4.9	7.9
72 hours...	Bacteria per seed	390,000	520,000	750,000	1,100,000	3,900,000
	Nodules per plant	7.8	6.2	6.0	3.0	4.9
10 days...	Bacteria per seed	100,000	175,000	90,000	330,000	1,700,000
	Nodules per plant	3.9	2.8	3.3	7.1	3.7
30 days...	Bacteria per seed	Less than 100	15,000	100,000	210,000	900,000
	Nodules per plant	4.1	5.1	1.9	3.6	5.7
90 days...	Bacteria per seed	10,500	1,700	Less than 100	2,750,000	2,000,000
	Nodules per plant	0.3	0.4	0.1	1.0	0.2
6 months.	Bacteria per seed	Less than 100	Less than 100	Less than 100	3,100,000	1,800,000
	Nodules per plant	3.5	8.0	1.5	8.0	11.0
9 months.	Bacteria per seed	Less than 10	Less than 100	Less than 100	1,500,000	1,400,000
	Nodules per plant	0.8	4.5	5.0	6.0	4.0

* Modified Ashby's agar 10 days at 25°C.

† 30 days growth in greenhouse.

Table 1 shows that the Early Brown variety of soybean, when placed in an infusion of nodules for 2 minutes and subsequently dried and stored, retained viable organisms on the seedcoat for over 3 months. Those soaked 20 minutes and 1 hour retained the organism 6 months, and those kept in the infusion

as long as 6 to 24 hours had many viable nitrogen-fixing bacteria on their seedcoats after 9 months. In general the bacteriological results were in agreement with the vegetation experiments carried on in the greenhouse for the purpose of observing nodule production. It was found in general that the greater the number of organisms found on the seeds, the greater was the number of nodules per plant in the pot experiments.

There were several notable exceptions to this generalization, however. Several pots were planted and treated in exactly the same manner as the test pots, with the exception that they were well inoculated after planting with a rich nodule infusion. It was thought that this might give an indication of the efficiency of nodule production by the bacteria on the seeds being tested. The pots inoculated in this way gave, on an average, about thirty per cent more nodules than test plants. The data are not conclusive, hence no deductions are drawn. It is probable, however, that more nodules were produced for the reason that more nodule-producing bacteria were present. There were some discrepancies in the results, especially in the pot experiments, for example—Early Brown soybean treated 20 minutes and stored for 3 months gave an average of but 0.4 nodule per plant. After 6 months there was an average of 8 nodules per plant. In another case beans treated 6 hours produced but 1 nodule per plant after 3 months, while after 6 months it produced 8. What caused such fluctuations is not known, but that they are often met with in microbiological work is not new to research workers.

On the whole, then, the preliminary experiment shows that soybeans may be inoculated, if ordinary precautions against contamination are used and stored for periods of 3 months, without seriously affecting the viability of the organisms upon the seedcoats. It is not recommended that seeds be treated with an inoculant for periods over one hour, as the germinating power may be seriously injured. In view of these interesting results a more thorough investigation was planned and carried out as here described.

MAIN EXPERIMENT

Both soybean and alfalfa seed were used in this experiment. The variety of soybean used was the Black Eyebrow, while the common variety of alfalfa, grown in Arizona, was used. The portion of the tests dealing with soybeans will be taken up first, the plan of the experiment being briefly as follows:

1. Soybean seeds were treated with a nodule infusion for varying lengths of time, then removed, dried, stored and tested at fixed periods for the presence of *B. radicicola*. The test consisted of two parts, one bacteriological and the other vegetation, that is the seeds were planted in pots in the greenhouse and nodule formation observed.

2. This experiment was the same as no. 1, except that the seeds were treated after inoculation with a sterile 10 per cent gum tragacanth jelly.

3. Soybean seeds were treated for a varying length of time with a commercial culture of *B. radicicola*. The seeds were then dried, stored and

tested at fixed periods of time for the presence of the nitrogen-gathering organisms.

4a. Moist soybean seeds were inoculated with air dry infected soil, and tested after fixed periods for *B. radicicola.*

b. Soybean seeds were inoculated with a nodule suspension for 10 minutes and subsequently dusted with air-dry sterile soil. The purpose of experiment 4a and 4b was to determine whether soil either moist or air-dried would not aid in lengthening the viability of the organisms upon the seedcoats.

TABLE 2

Results of bacteriological and vegetation experiments with soybean seed inoculated with soybean nodule infusion

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	6 hours
1 hour	Bacteria per seed	245,000	4,000,000	2,000,000	2,300,000
	Nodules per plant	1.0	4.3	1.0	1.3
6 hours	Bacteria per seed	600,000	2,000,000	1,300,000	2,000
	Nodules per plant	1.0	1.3	4.0	1.3
24 hours	Bacteria per seed	8,000	250,000	580,000	94,000
	Nodules per plant	0.8	1.0	1.0	0.8
6 days	Bacteria per seed	55,000	85,000	205,000	50,000
	Nodules per plant	6.3	1.5	2.8	1.7
15 days	Bacteria per seed	20,000	80,000	90,000	18,000
	Nodules per plant	7.0	6.2	1.5	0
30 days	Bacteria per seed	4,000	95,000	450	20,000
	Nodules per plant	1.3	11.0	3.0	3.3
90 days	Bacteria per seed	Less than 100	600	330,000	1,850
	Nodules per plant	3.5	7.0	8.0	5.3
6 months	Bacteria per seed	2,000	9,500	2,700	1,100
	Nodules per plant	4.0	6.0	No plants	7.0

PROCEDURE

Procedure for the bacteriological test was the same as that used in the preliminary experiment. Mercuric bichloride, 1 to 500, for 5 minutes was used to sterilize the seeds, 5 of which were used in each bacteriological test made. The seeds were dried on sterile paper towels, in a quiet, dark room and afterwards stored in the dark, in sterile paper bags. Modified Ashby's medium was used throughout the experiment. The dilutions most commonly used were 1: 1,000, 1: 10,000 and 1: 100,000, although occasionally plates were

poured at higher or lower dilutions, especially if it was found that the number of cells present was extremely high or low.

The methods of testing nodule production in pots were practically the same as those already described in the preliminary experiments. A coarse red sandy soil, of very low fertility, was used in the pots. This sand came from a pit and was found negative several times for all types of *B. radicicola*. In each pot were placed 2 pounds of sand, to which had been added 1 gm. of

TABLE 3

Results of bacteriological and vegetation experiments with soybean seed treated with gum tragacanth and inoculated with nodule infusion

Experiment 2a

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH CULTURE			
		5 minutes	30 minutes	1 hour	6 hours
1 hour	Bacteria per seed	1,180,000	2,460,000	2,900,000	3,250,000
	Nodules per plant	0.5	1.3	2.2	1.5
6 hours	Bacteria per seed	850,000	450,000	2,850,000	105,000
	Nodules per plant	6.0	2.0	0.7	1.1
24 hours	Bacteria per seed	610,000	100,000	600,000	550,000
	Nodules per plant	1.3	1.0	2.4	5.1
6 days	Bacteria per seed	185,000	260,000	220,000	2,000
	Nodules per plant	4.4	6.2	1.4	2.1
15 days	Bacteria per seed	2,000	300,000	90,000	10,000
	Nodules per plant	11.0	2.6	1.0	1.0
1 month	Bacteria per seed	28,000	195,000	50,000	32,000
	Nodules per plant	14.0	2.9	2.5	3.7
3 months	Bacteria per seed	1,500	50,000	40,000	3,000
	Nodules per plant	3.2	5.2	1.0	13.0
6 months	Bacteria per seed	7,000	30,000	2,000	Less than 100
	Nodules per plant	8.1	4.6	7.6	7.2

potassium chloride, 1 gm. of acid phosphate and 2 gm. of calcium carbonate. All pots were sterilized in an autoclave at 10 pounds pressure for 20 minutes. Nodule counts were made after 30 days, but in some of the later experiments, this time was allowed to lapse a few days. It is not believed that this affected the results seriously. The results of the soybean experiments are given in tables 2 to 5.

The soybean nodule infusion used to inoculate the seeds contained 4,000,000,000 cells per cubic centimeter. It was made up by crushing fresh, well-

cleaned nodules in sterile water and filtering the liquid through 4 thickness of cheesecloth. The alfalfa-nodule infusion was prepared in the same way, but owing to the difficulty of obtaining nodules, the cells per cubic centimeter, numbered but 27,000,000. As a result of several tests both the soybean and alfalfa seeds used in the work were shown not to harbor any member of the *B. radicicola* group. Check pots planted from time to time also showed the soil used, to be negative for this organism.

TABLE 4

Results of bacteriological and vegetation experiments with soybeans and alfalfa seed inoculated with commercial cultures

Experiment 3

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH CULTURE			
		Soybeans		Alfalfa	
		5 minutes	1 hour	5 minutes	1 hour
6 hours	Bacteria per seed	100,000	3,000,000	812,000*	2,220,000
	Nodules per plant	2.8	19.0	12.5	12.0
72 hours	Bacteria per seed	490,000	1,900,000	170,000	280,000
	Nodules per plant	2.4	6.5	6.4	9.5
15 days	Bacteria per seed	60,000	530,000	360,000	800,000
	Nodules per plant	0	2.1	3.4	4.0
30 days	Bacteria per seed	27,000	70,000	650,000	50,000
	Nodules per plant	2.8	6.9	4.7	2.6
3 months	Bacteria per seed	3,000	58,000	430,000	24,000
	Nodules per plant	11.6	3.0	6.0	4.5
6 months	Bacteria per seed	500	5,000	18,000	2,000
	Nodules per plant	8.0	4.9	4.4	7.7

NOTE—The original soybean culture contained 91,000,000 cells per cubic centimeter while the alfalfa culture contained 600,000,000.

* Bacteria per gram of alfalfa seed.

DISCUSSION OF RESULTS

The data incorporated in tables 2 and 3 show that soybean seeds kept in contact with the inoculant for periods of time varying from 5 minutes to 6 hours, retain viable organisms on their seedcoats for at least 6 months. The greatest decrease in the number of organisms per seed took place during the first 6 days. In fact, the cells began to perish from the first hour. Those cells of low viability and those most exposed were undoubtedly the ones that died first, while the more resistant ones and those better protected, remained alive longest. There is but little correlation between the number of bacteria

per seed and the nodules produced per plant. From the data it appears that "physiological efficiency" (a term first suggested by Dr. J. G. Lipman) of the bacteria plays an important rôle in nodule production. It seems that those organisms which are most resistant to drying are those which are able to penetrate the root hairs of the plants and set up a symbiosis. It is probable that a great many living cells do not multiply on agar plates, hence this may account for the production of nodules from seeds showing very few cells. It

TABLE 5

Results of bacteriological and vegetation experiments with soybean and alfalfa seeds treated with air-dry infected soil

Experiment 4

STORAGE PERIOD		METHOD OF TREATMENT			
		Soybeans		Alfalfa	
		Moist inoculated seeds dusted with air-dry soil	Moist sterile seeds dusted with infected soil	Moist inoculated seeds dusted with air-dry soil	Moist sterile seeds dusted with infected soil
6 hours	Bacteria per seed	720,000	266,000	Lost	Lost
	Nodules per plant	7.7	8.2	8.5	5.8
72 hours	Bacteria per seed	Less than 100	100,000	180,000*	1,000,000
	Nodules per plant	2.8	4.5	1.7	4.2
15 days	Bacteria per seed	50,000	168,000	2,000	250,000
	Nodules per plant	1.1	1.0	4.1	3.9
30 days	Bacteria per seed	10,000	20,500	13,000	35,000
	Nodules per plant	0	6.0	0.8	3.4
3 months	Bacteria per seed	Less than 100	230	8,000	66,000
	Nodules per plant	9.0	4.9	5.0	1.9
6 months	Bacteria per seed	Less than 100	50	3,500	2,000
	Nodules per plant	4.2	5.4	3.0	0.9

NOTE—The soybean soil contained 14,000,000 *B. radicicola* per gram, while the alfalfa soil contained 6,600,000 cells per gram.

* Bacteria per gram of alfalfa seed.

was thought that this experiment might thrown some light upon the relation of the bacteria developing on agar plates and those producing nodules on plants.

It is certain that only a very few cells have the power to infect root hairs. Oftentimes seeds showing over a million bacteria when planted and grown under suitable conditions produced but one or two nodules per plant. The ratio, then, of the number of bacteria capable of developing on agar plates

to those capable of producing nodules is an exceedingly narrow one. Further study of this problem is sorely needed, especially studies which might show the factors influencing infectibility of the cells. It is impossible to explain in the light of our present knowledge of the subject, certain of the data. For example, in table 1, soybean seeds which have been soaked for 6 hours in the inoculant and which showed 3,250,000 bacteria per seed, gave but 1.3 nodules per plant, while similar seeds 6 months later showed less than 100

TABLE 6

Results of bacteriological and vegetation experiments with alfalfa seed inoculated with alfalfa nodule infusion

Experiment 1b

STORAGE PERIOD		LENGTH OF TIME SEED IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	6 hours
1 hour.....	Bacteria per gram of seed	250,000	160,000	900,000	570,000,000
	Nodules per plant	4.7	3.1	7.0	2.1
24 hours.....	Bacteria per gram of seed	2,400,000	113,000	3,000,000	440,000,000
	Nodules per plant	7.6	3.3	3.2	3.5
6 days.....	Bacteria per gram of seed	3,750	5,800	2,070,000	8,000,000
	Nodules per plant	3.2	2.8	3.3	3.4
15 days.....	Bacteria per gram of seed	12,000	70,000	156,000	36,000,000
	Nodules per plant	0.4	1.7	0.3	1.1
1 month....	Bacteria per gram of seed	3,000,000	14,000	7,500	28,000,000
	Nodules per plant	3.2	3.1	2.3	2.3
3 months...	Bacteria per gram of seed	2,500	600*	4,000	2,500,000
	Nodules per plant	4.3	7.9	9.0	5.4
6 months...	Bacteria per gram of seed	53,000	5,000*	2,000	2,000,000
	Nodules per plant	4.0	0.8	4.1	4.9

* Poor plates.

cells per seed and produced an average of 7.2 nodules per plant! If there were any disturbing factors in the experimental work, the writer is not aware of them, as all ordinary precautions against contamination were taken.

In the case of seeds coated with gum tragacanth, the results are similar to those already recorded. The gum-coated seeds did not give sufficient protection to the bacteria to cause increased nodule production on the plants. The addition of gum, glue or even milk has been advocated by various persons to increase the longevity of the nitrogen-producing bacteria on legume seeds. This experiment indicates that the use of such substances is of doubtful value. As in the first experiment in every case bacteria survived on the seeds

for at least 6 months and were able to produce nodules on the roots of plants grown from these seeds. In this experiment also the number of bacteria per seed and the number of nodules produced per plant did not go together in general.

In table 4 are recorded the results of the inoculation of soybean and alfalfa seed with commercial cultures. The commercial culture used was Mulford's Nitrogerm in both cases. The directions were followed, the seeds being allowed to stand in the culture, for 5 minutes in one case and 1 hour in the

TABLE 7

Results of bacteriological and vegetation experiments with alfalfa seeds treated with gum tragacanth and inoculated with nodule infusion

Experiment 2b

STORAGE PERIOD		LENGTH OF TIME IN CONTACT WITH INOCULANT			
		5 minutes	30 minutes	1 hour	8 hours
6 hours	Bacteria per gram of seed	350,000	210,000	170,000	Lost
	Nodules per plant	9.0	6.0	12.5	4.3
24 hours	Bacteria per gram of seed	335,000	2,220,000	10,000,000	33,000,000
	Nodules per plant	6.6	5.8	5.5	4.8
6 days	Bacteria per gram of seed	260,000	1,720,000	760,000	14,400,000
	Nodules per plant	2.9	3.4	2.3	3.1
15 days	Bacteria per gram of seed	120,000	120,000	Lost	8,000,000
	Nodules per plant	1.5	2.0	4.2	3.8
1 month	Bacteria per gram of seed	720,000	130,000	2,000,000	7,200,000
	Nodules per plant	5.4	1.8	4.1	4.1
3 months	Bacteria per gram of seed	1,500	36,000	520,000	3,000,000
	Nodules per plant	6.0	11.8	11.3	8.6
6 months	Bacteria per gram of seed	No test	300,000	150,000	No test
	Nodules per plant	5.9	3.3	4.4	4.0

second case. Here the counts are much more uniform than those recorded in the previous experiments. The rate of dying out of the cells was much more uniform. This rate was most rapid in the first few hours, but after this it became more gradual, until at the end of 6 months the average number of bacteria per seed for both soybean and alfalfa was 6370, while the average number of nodules produced per plant was 5.5. These data show that *B. radicicola* withstands drying fairly well. This is not in accord with the experience of several other investigators. In this work, however, the initial inoculation was very rich and although the number of bacteria surviving for long periods of time was not great, yet those which did survive seemed to

retain their nodule-producing powers to a large extent. In case commercial cultures are used to inoculate seed it should be made certain that these cultures are rich in viable bacteria to insure living organisms remaining upon the seed-coats for a long time.

Referring to table 5, where data regarding seed inoculation by the use of soil are recorded, it is seen at a glance that under the conditions of the experiment soil did not aid in preserving the life of the bacteria. However, as in the other tests, viable cells persisted on the seed for over 6 months and, moreover, were able to produce nodules on the young seedlings. At the end of 30 days there was still an average of about 15,000 viable cells per soybean seed and about 25,000 cells per gram of alfalfa seed. The results are similar in the case of both of these legumes. Of the two inoculation methods tested out, it appears to be much more efficient to treat the moist uninoculated seed with air-dry infected soil than to treat the moist inoculated seed with sterile air-dry soil. The soil used to inoculate the soybeans contained 14,000,000 bacteria per gram, while the alfalfa soil contained 6,600,000 per gram. The soybean soil, when plated after 30 days, showed 70,000 *B. radicicola* per gram, while the alfafa soil showed none. The soil used in this experiment was a fine Penn loam, which had been sterilized by heat previous to inoculation with *B. radicicola*. In the tests, in which alfalfa seed was used, the experimental procedure was the same as with the soybeans. For the bacteriological tests, glass tubes containing 1 gm. of seed were used in place of individual seeds as was done in the soybean tests. This was done because of the small size of the alfalfa seed and also to reduce the experimental error.

Turning to tables 7 and 8, one sees that the results obtained by drying the alfalfa organism, under various conditions, are in accord with those already found to be true with the soybean bacteria. There does not seem to be much difference in the resistance of these two varieties of *B. radicicola* as regards desiccation. Although more bacteria are present on seeds soaked for a long time in the infusion, yet in general, the shortest period of time the seeds were kept in contact with the inoculant (i.e. 5 minutes), gave nearly as good results as longer periods of time. This was also found to be true by and large in the case of soybeans inoculated with either nodule infusion or commercial cultures. It is recommended, therefore, that 10 minutes is sufficient time to allow seeds to remain in contact with the inoculant. Seeds soaked over one-half hour and subsequently dried have their germinating power somewhat impaired. This was shown to be the case, when both soybean and alfalfa seeds were kept in contact with the liquid for over half an hour. The germination of the soybeans was injured more than that of the alfalfa.

In the case of alfalfa seed treated with gum tragacanth, the results obtained do not justify the use of such substances. It probably aids to some extent in protecting the bacteria, but not enough to justify the cost and trouble of using it. The data contained in these two tables show that the nodule organism is capable of withstanding considerable desiccation, and that the

inoculated seeds were able to produce plants having abundant nodules, when planted at fixed periods up to and including 6 months.

During the progress of the experiment, it was decided to reinoculate with a rich nodule infusion some of each series of pots. Theoretically, at least, this should give important data regarding the value of the two methods of inoculation, as well as whether the addition of a great number of bacteria would produce more nodules per plant than fewer cells. The data, as compiled in table 8, show the proportion of nodules produced in pots where the number produced as a result of inoculated seed alone, is taken as unity, compared with the nodules produced by both inoculated seeds and inoculated soil. Both soybeans and alfalfa were used.

In the soybean tests it is seen that the proportion of nodules produced from the inoculated seeds alone to the soil plus seed method was fairly constant. The average figure is 1: 2.9. That is, the addition of inoculating material to

TABLE 8

Proportion of nodules produced in pots where inoculation by seed alone is taken as unity and compared with inoculation by both seed and soil

LENGTH OF STORAGE OF INOCULATED SEEDS BEFORE PLANTING	NUMBER OF POTS IN TEST		PROPORTION OF NODULES PRODUCED BY INOCULATED SEED ALONE TO INOCULATED SEED + INOCULATED SOIL	
	Soybean	Alfalfa	Soybean	Alfalfa
1–24 hours	6	20	1: 2.4	1: 1.2
48 hours	1	3	1: 1.8	1: 1.3
6 days	4	12	1: 1.7	1: 1.0
15 days	4	8	1: 2.1	1: 1.5
3 months	5	5	1: 3.7	1: 0.8
6 months	3	2	1: 1.8	1: 3.9

soil produced more nodules per plant than where inoculated seeds alone were used. The length of storage did not appear to make a very great difference in this proportion.

In the case of alfalfa, the proportion is narrower, i.e. 1: 1.7. Here also the addition of inoculating material to the pot caused a proportionate increase in the number of nodules. Therefore, with both alfalfa and soybean seeds, the number of nodules per plant is increased by inoculating the pots with a nodule infusion in addition to planting inoculated seeds. These data are somewhat contradictory to those obtained in the previously reported experiments, where it appeared that numbers of bacteria did not seem to influence the nodule production greatly.

SUMMARY

The following statements seem justified as a result of the experiments here described.

1. Seeds may be satisfactorily sterilized by the use of mercuric chloride, 1: 500 kept in contact with the seed for 5 minutes.

2. The methods herein described for carrying on bacteriological and vegetation experiments for testing the viability of *B. radicicola* on seeds, give very good results.

3. Soybean or alfalfa seeds inoculated with a nodule infusion retain viable organisms on their seedcoats 6 to 9 months. Plants grown from these seeds had nodules on their roots, showing that the bacteria had not lost the infecting power.

4. The soybean and alfalfa nodule organisms seem to be able to withstand nearly the same degree of desiccation.

5. The shortest period of time, i.e. 5 minutes, that the seeds were kept in contact with the inoculant gave nearly as good results as longer periods of contact.

6. Gum tragacanth and probably similar substances do not aid greatly in keeping alive the bacteria upon the seeds. Their use is not recommended.

7. Infected dry soil, dusted upon moist seeds, gave better results than sterile, dry soil dusted upon moist inoculated seeds. The seeds inoculated with soil did not retain *B. radicicola* longer on their coats, neither did the plants have a greater number of nodules, than where nodule infusion or commerical cultures were used.

8. Commercial cultures, when used to inoculate soybeans or alfalfa, give as good results as nodule infusion.

9. In general the number of bacteria per seed and the number of nodules per plant did not correlate closely.

10. It appears that but an exceedingly small percentage of the cells present on a seed are able to produce nodules on the roots of the seedling. This ratio may be as narrow as 1: 1,000,000.

11. It seems probable that those cells which resist desiccation longest are those which have the greatest infectibility.

12. The period which showed the greatest destruction of cells was the first few hours after inoculation; after this the cells perished relatively more slowly, and uniformly.

13. The inoculation of soil in pots by means of a rich nodule infusion increases considerably the number of nodules produced per plant over the number produced by inoculated seed alone.

14. It is not recommended that inoculated seeds be stored for long periods of time before planting, but ordinarily a delay of several days, or even a month should do no great harm. There seems to be little difference in the resistance to desiccation whether nodule infusion, commerical cultures or soil be used as the infecting agent.

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SOME NOTES ON THE CAUSE OF THE UNPRODUCTIVITY OF "RAW" SUBSOILS IN HUMID REGIONS

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In a recent paper, Harmer (3) has reported vegetation experiments showing that some Minnesota subsoils are as productive towards alfalfa when inoculated as are the corresponding surface soils, while others are much less productive and hence may properly be considered "raw." His experiment involved no attempt to determine the cause of the unproductivity where it existed, and, as he was unable to continue the study, the writer has used the remaining portions of the bulk sample which he had collected at two of the places, viz. Canby and Renville.

The possibility of a deficiency of available potash or phosphoric acid or both as the cause of the "rawness" in such cases has been discussed by Lipman (4, p. 290), Alway (1) and Alway, McDole and Rost (2, p. 10).

The object of the experiment described below was to determine whether the addition of potash and phosphoric acid would fully eliminate the unproductivity.

EXPERIMENTAL

The origin and method of collecting the bulk samples and their properties have been fully dealt with by Harmer, and it will suffice here to state that Canby and Renville are in the prairie region of west central Minnesota and that the soils were taken from pits in virgin prairies and shipped to the experiment station in the autumn of 1916. The portions used in the experiment had remained in sacks until the fall of 1917. The properties of the soils are reported in table 1 (3, p. 395).

Owing to the limited quantity of the soils remaining, it was not possible to carry out the pot experiments in duplicate, but as Harmer (3, p. 400) has shown, the use of single pots may be expected to lead to the same conclusions as do duplicates.

The 4 air-dried soils, both surface and subsoil, were prepared by mixing and passing through a coarse screen. Half of each was placed directly in a vegetation vessel, while the other half was first treated with disodium phosphate and potassium sulfate, each in amounts equivalent to 1000 pounds per acre. The soils were spread out upon large sheets of oilcloth, the powdered

salts sifted over them and the whole thoroughly mixed and transferred to the vegetation vessels. These were of galvanized iron 12 inches deep with a surface area of 0.75 square feet and painted on the inside with pitch tar, drainage being effected by holes in the bottom and a layer of coarse gravel. The treatments are shown in table 2. The soils were brought to approximately the moisture equivalent and the pots allowed to stand 48 hours, and, on December 3, 1917, planted to alfalfa. When the plants were up and several inches in height they were thinned to leave 30 plants in each pot. The inoculation had already been assured.

In all, 5 crops were harvested, the first on April 25, the last on November 20. The plants on the untreated subsoils were spindling and without much foilage, although their color was as good as any. In table 2 the yields are reported. The increases due to the treatment are shown, it being assumed for the purpose that the yield on each of the unfertilized soils was 100. It will be seen that in the case of the two surface soils which received no treat-

TABLE 1

Composition and properties of the soils used

LOCATION	DEPTH	MOISTURE EQUIVALENT	NITROGEN	ORGANIC CARBON	ORGANIC MATTER*	REACTION OF THE SOIL
			per cent	*per cent*	*per cent*	
Canby	1 to 6 in.	30.25	0.329	4.015	6.922	Neutral
	Third foot	23.24	0.051	0.770	1.328	Neutral
Renville	1 to 6 in.	35.91	0.393	5.134	8.852	Neutral
	Third foot	27.28	0.051	0.865	1.491	Neutral

* Computed from organic carbon. Organic matter = Organic carbon × 1.724.

ment the yields were very similar, amounting to totals of 60.5 gm. for that from Canby and 62.4 gm. for that from Renville, equivalent to almost 4 tons of hay per acre. The applications of potash and phosphoric acid caused appreciable increases in the yields on these surface soils, 28 per cent in the case of that from Renville and 39.1 per cent with that from Canby. On the unfertilized subsoils the yields were very low, only one-fifth to one-third as high as on the corresponding surface soils. In the case of the fertilized subsoils, however, there was a very great improvement in growth, the appearance of the plants was exceptionally good and much higher yields were obtained at each harvest, even better than on the unfertilized surface soils, the total for the 5 crops being equivalent to over 4 tons of hay per acre compared with 0.85 tons on the unfertilized. The lowest increase noted was in the case of the Canby subsoil at the time of the cutting of the fourth crop, viz. 160.6 per cent, while the highest was with the first crop on the Renville soil which gave over 1150.0 per cent increase. For the 5 crops the increase for the Renville subsoil was 472.1 per cent and for the Canby subsoil 309.3 per cent. From these data it is to be seen that by the additions of readily available potash

and phosphoric acid the "rawness" of the subsoils, as evidenced by the growth and yield, had been eliminated, the alfalfa making as vigorous a growth and giving as high a yield as on the corresponding surface soils which had received no fertilizer.

TABLE 2

Yields of dry matter from 5 crops of alfalfa

SOIL	TREATMENT	FIRST CROP APRIL 25	SECOND CROP MAY 24	THIRD CROP JUNE 26	FOURTH CROP AUGUST 1	FIFTH CROP NOVEMBER 20	TOTAL FOR 5 CROPS	TOTAL FOR 4 CROPS IN EARLIER EXPERIMENT*
		Renville						
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Surface 6 in.	Nothing	4.0	12.9	21.1	14.3	10.1	62.4	70.3
Surface 6 in.	Potash and phosphoric acid	5.9	14.0	24.9	18.8	15.3	79.9	
	Increase (per cent)	47.5	8.5	18.0	31.4	51.5	28.0	
Third foot..	Nothing	0.2	2.9	3.7	3.4	1.3	11.5	15.3
Third foot..	Potash and phosphoric acid	2.5	16.7	18.1	16.6	11.9	65.8	
	Increase (per cent)	1150.0	475.5	389.2	388.2	815.3	472.1	
		Canby						
Surface 6 inches..	Nothing	4.5	13.5	17.5	14.6	10.4	60.5	51.8
Surface 6 inches..	Potash and phosphoric acid	3.2	20.3	23.8	19.6	17.3	84.2	
	Increase (per cent)		50.3	36.0	34.2	66.3	39.1	
Third foot..	Nothing	0.2	3.1	5.2	6.1	2.6	17.2	19.8
Third foot..	Potash and phosphoric acid	1.6	14.9	24.7	15.9	13.3	70.4	
	Increase (per cent)	700.0	380.6	375.0	160.6	411.5	309.3	

* Harmer (3, p. 398).

The above described experiment proves that the "rawness" shown to exist in some subsoils may be removed by an application of potash and phosphoric acid, and hence in them it is probably due to a deficiency of available mineral nutrients.

SUMMARY

Vegetation experiments with certain Minnesota prairie subsoils previously found "raw" towards inoculated legumes have shown that an application of soluble potash and phosphoric acid fertilizers removes this infertility, rendering the subsoils as productive as the corresponding surface soils, evidence that "rawness" in these cases is due to a lack of readily available mineral nutrients.

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THE EFFECT OF SOYBEAN GERMINATION UPON THE GROWTH OF ITS NODULE-FORMING BACTERIA[1]

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The study of the influence of plants upon soils is becoming quite as important as that of soils upon plants (14). It is well known that certain crops require the presence of a particular kind of bacteria in the soil in order to yield the largest return. When the proper nodule-forming bacteria are entirely absent from the soil or are present in relatively small numbers, it is customary to inoculate, i.e., to add the bacteria. Inoculation may be carried out by one of two ways: by the transfer of soil containing the particular kind of bacteria or by the less laborious method of sowing inoculated seed.

It has been shown that plants exert an appreciable influence upon the number of bacteria in soil (11). The question may be raised as to whether or not the first stages of the plant's active growth or, to be more specific, whether or not the germination of legume seed, has an injurious effect upon the growth of the nodule-forming bacteria with which the seed have been inoculated.

In an extensive series of investigations, Hiltner and Störmer (10) have pointed out that direct inoculation of large seeds such as lupine and soybean, may not lead to nodule formation and have concluded that the failure to secure inoculation was due to the excretion of substances from the seedcoats during the process of swelling. In their investigations, soybean seed were covered with distilled water for 5 hours and the water extract was used in studying the growth of the bacteria and their transformation into bacteroids.

As a result of their studies upon nodule bacteria in aqueous extracts of seedcoats and of the contents of legume seed, they conclude that a protein is given off from the seed contents that favors the growth of the nodule bacteria, whereas from the seedcoats substances are given off that may help to plasmolyze and destroy the bacteria. They suggest the possibility of antagonism between the substances given off by the seed contents and by the seedcoats that reduces the toxic action of the seedcoat excretion upon the bacteria. It seemed important, therefore, to investigate the toxicity of certain legume seed during their germination, for the nodule bacteria inoculated upon the seedcoat.

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The method of experimentation consisted in adding a water suspension of the nodule bacteria to bacteria-free seed in a Petri dish, followed by sterilized Ashby's agar (1). The plates were rotated after the addition of the bacterial suspension, as well as after the addition of the agar. The former rotation insured the inoculation of the seed, whereas the latter rotation distributed the remainder of the bacteria uniformly throughout the plate. The uniform distribution of the bacteria made it possible to observe more readily any toxic effects upon the growth of the bacteria. The seeds were then distributed in the agar by means of a sterilized platinum needle.

The Ashby agar (7) contained sucrose in the place of mannitol, the dibasic instead of monobasic potassium phosphate, and no calcium carbonate. The seed used, unless otherwise specified, were medium yellow soybeans. The sobyean nodule bacteria were obtained from a growth on Ashby sucrose agar. Several loopfuls of the culture were added to a flask containing a small volume of sterilized distilled water and after thorough shaking, transfer of one cubic centimeter of the suspension to the plates was effected by means of sterile pipettes.

Sterilization of the seed was at first carried out by means of the autoclave with the consequent death of the cells. Wisconsin black, medium yellow, and medium early green soybean seed were sterilized in the autoclave in tubes and then sown in plates as described. If substances that are given off by the dead seed exert a toxic action upon the growth of the nodule bacteria, we should expect to observe an inhibition in the growth of the bacteria about the seed. In no case was an inhibition of the growth of the bacterial colonies apparent, although it was difficult to determine the relative number and size of the colonies near the seed, due to the diffusion of opaque substances from the dead seed. It is possible that heat sterilization destroyed the toxic substances if such were present in the living seed, although previous investigators (10) sterilized the water in which the soybean seed had been soaking.

The seedcoats of the three varieties of soybean seed were extracted with water and the extract sterilized in the autoclave. The extracts were added to sterile pieces of quantitative filter paper upon Ashby's sucrose agar containing a uniform inoculation of nodule bacteria. After a week's incubation of the plates at 27°C. there was no inhibition of growth of the colonies about or beneath the paper. The extract of the Wisconsin black soybean seed, after being autoclaved, still possessed the properties of an indicator of hydrogen-ion concentration.[2] The other two varieties did not contain an indicator in their seedcoats.

[2] It may be of interest at this point to describe the indicator that was found in the Wisconsin black soybean seed. The seedcoats were extracted with 50 per cent alcohol for 5 hours at 30°C. and gave a wine red solution. The same amount of indicator (5 drops) was placed in tubes containing equal quantities of buffer solutions of known P_H value. The color changes were mostly of the gradual transition type in going from P_H 1–10, being pink to red on the more acid side of the neutral point for distilled water and from pink-gray to

The seedcoats of the three varieties of soybean seed were removed, and the seedcoats and the seed contents of each variety sterilized in separate tubes in the autoclave. When placed on the usual medium, as described, and incubated for a week, no inhibition of the growth of the colonies of nodule bacteria was apparent.

It was desirable to sterilize the soybean seed in such a way as to eliminate the use of the autoclave which killed the cells. That sterilization (16) offered considerable difficulty is readily understood. Here it is essential not only to rid the seed of any adhering microorganisms, but also to take precautions not to replace such organisms by a sterilizing agent that is harmful to the bacteria that are to be studied. To this end it will be helpful to mention the observations upon the sterilization of yellow soybean seed by the use of a 0.25 per cent solution of mercuric chloride (15).

The sterilization apparatus was essentially that described by Fred (7). It consisted of a series of milk bottles connected by means of glass tubing with rubber connections and clamps. The apparatus was a closed system consisting of several empty bottles, a bottle of distilled water, and a bottle of mercuric chloride. It was then mounted for convenience on a board and sterilized in the autoclave. During the process of seed sterilization, a water suction pump was used to exhaust some of the air from the system. The seeds were covered with the mercuric chloride solution for seldom over 3 minutes (the time being regulated according to the type of seed used), and then were repeatedly washed under reduced pressure with sterilized distilled water, after which they were transferred to a sterile dish and were ready for use.

Sterile Petri dishes, containing the $HgCl_2$-sterilized yellow soybean seed, aqueous suspension of soybean bacteria, and Ashby's sucrose agar, were incubated for about a week (5 to 8 days) at 27°C. and the distribution and growth of the colonies observed in relation to the germinating seed. In order to show more clearly any injurious effects that the substances excreted from the seed might have upon the growth of the bacteria, the suspension of bacteria was heavily seeded with the nodule bacteria. The Petri dishes in some experiments were placed upright, while in others they were inverted; however, the position was found to have no effect on the results.

When the yellow soybean seeds were sterilized by the mercuric chloride method and were sown in the manner described, the condition shown in plate 1, figure 2, was observed. The plate had been inverted in order to permit the rootlets to grow in the moist chamber beneath the medium, and, for the purposes of photography, the germinated seeds were removed from the plates.

yellow-brown on the alkaline side. Between P_H 6 and 8, it was possible to detect color differences for each 0.2 change in P_H, the most marked changes being from about P_H 7–7.6. After the extract had been sterilized in the aotoclave and was left standing for about two weeks, the indicator lost many of its properties, in that the different buffer solutions gave only varying degrees of yellow or brown color.

It will be noted that a zone extends about each cavity in the medium where the seeds had been imbedded. The plates showed clear and transparent zones, whereas the remainder of the medium had somewhat of an opaque appearance. Under the microscope the clear zone was found to be entirely devoid of colonies. At the outer boundary of the clear zone the colonies were as numerous as anywhere else in the medium. The limiting line, up to which the colonies could still grow, was sharply defined and at this line there appeared to be a piling up of the colonies, possibly a region of stimulating concentration of the mercuric chloride. When medium early green and also Wisconsin black soybean seeds were used, similar results were obtained.

The question naturally suggested itself, are such zones due to the excretion of a substance by sterilized seed that is toxic to the growth of soybean nodule bacteria? In order to control the experiments, small squares of unhardened quantitative filter paper were sterilized with the soybean seed, using the mercuric chloride method and were subsequently treated as though the paper were actual soybean seed.

Plate 1, figure 1 shows the result of the experiments using such filter paper as described. The soybean seed, sterilized with the filter paper, gave results similar to those shown in plate 1, figure 2. While there were no such sharply demarcated clear zones about the pieces of filter paper, there were some areas near the filter paper that were unaffected, other areas that had a reduced number of colonies, and still other areas possessing no colonies whatsoever, but showing a more gradual transition to an increasing number of colonies farther away from the paper. The filter paper must have retained some (however small a quantity) of the mercuric chloride in the sterilization process, some of which it later gave up to the medium.

It was a simple matter, then, to ascertain whether or not the giving off of mercuric chloride by the seed or by the filter paper (that was sterilized with the seed) was responsible for the observed clearing of the medium. A sterile platinum loop bearing a thin film of mercuric chloride solution such as was used in the sterilization process, was touched against the medium at places marked upon the plates and after two days the plates were re-examined. Some of the plates that were used contained Ashby's sucrose agar alone, whereas others contained in addition very numerous nodule bacteria colonies. On both kinds of plates the touched areas were found to have cleared as in the clear zones of plate 1, figure 2. The clearing was due to the action of the mercuric chloride upon the Ashby sucrose agar. The presence of very numerous bacterial colonies about such zones tends to increase the contrast between the cleared and uncleared areas.

Evidently it is most difficult, if not impossible, in the sterilization of soybeans by the use of mercuric chloride solution with reduced pressure, entirely to remove the last trace of the mercuric chloride. The use (13) of mercuric chloride (3) as a means of sterilizing seed is a common practice. For many

purposes its use cannot be questioned, though it is not safe to assume that because the germination of the seed has not been injured, the mercuric chloride has been entirely removed.

Having found the use of mercuric chloride objectionable as a sterilizing agent (2, 12) for the soybean seed, other methods of sterilization were tried. Yellow soybean seeds were dipped (singly) a few seconds in concentrated sulfuric acid and immediately washed with large volumes of sterile distilled water under reduced pressure. This method is open to the objection that in dipping the seeds from the acid into the water, the temperature of the seedcoat cells is raised and perhaps the nature of the excreted substances changed. Some of the seeds were dipped in alcohol and rapidly flamed before being dropped into a sterile Petri dish. Other of the seeds were flamed directly. Such methods are open to the same criticism of undue heating and, even though germination was seldom affected, it was frequently difficult to secure seeds free of bacteria.

The writers found it desirable, in studying the effect of substances given off by germinating soybean seeds upon the growth of nodule bacteria, to remove all possible objections as regards the sterilization of the seed. Accordingly, yellow soybean pods, that were dry but intact, were secured. Some of the pods were carefully opened (8, 9) upon sterile dishes and the seed transferred to Petri dishes, after which the usual procedure of adding the bacteria and the medium was carried out.

As variants upon this method, some pods were dipped in alcohol, flamed, and the seed removed; other pods were opened and the seed dipped singly into alcohol and flamed. The seeds of some pods were given the sulfuric acid treatment, while the seeds of other pods were removed from the intact pods and transferred to the sterilizing apparatus (7) without the use of mercuric chloride or any other antiseptic agent, and were repeatedly and rapidly washed with large volumes of sterile distilled water under reduced pressure. The sterilization processes seldom required more than 3 to 5 minutes, the shortest possible time being used in every case in order not to cause any considerable leaching during the sterilization process.

When the plates secured from such seed had been incubated for 5 to 8 days at 27°C., it was found that in every instance, the nodule bacteria colonies were growing up to and about the germinating seed. In the neighborhood of the seeds there was a tendency for the colonies to increase both in size and in number, possibly due to the excretion of salts from the seed. In no case was there a clear zone or an inhibition to the growth of the colonies observed.

The sulfuric acid treatment or the flaming process did not give results that differed from the other methods of seed sterilization, but when carried on without the proper precautions there appeared visible effects upon the cotyledons. The seed that had been picked with sterile forceps from pods and had been repeatedly washed with sterile distilled water under reduced

pressure showed only an occasional contamination. When the same procedure was followed, except that the pods were momentarily dipped into alcohol and quickly drawn through the flame, no contamination was ever encountered.

Differences in susceptibility to inoculation between varieties of soybeans and even in some cases total resistance to inoculation, as had been reported by several investigators, has been shown by Leonard (13) not to exist in the 19 varieties of soybeans tested. The smoothness and oily nature of soybean seedcoats has been suggested (5) as possibly presenting some of the difficulties encountered in soybean seed inoculation. It might be mentioned in this connection (5) that frequently the difficulty in securing soybean nodules has been due to the inoculation of the seed with unreliable cultures. The distribution (4, 8) of tested cultures of nodule bacteria offers an effective control.

SUMMARY

It has been found that in studying the effect of soybean seed germination upon the growth of its nodule bacteria it is not permissible to use mercuric chloride as a sterilizing agent. In studies upon yellow soybean germination, it has been shown that the germination of the seed favors the growth of the bacteria. When bacteria-free soybean seeds were obtained directly from the pods and inoculated with soybean bacteria, no toxic action of substances excreted from the germinating seed upon the growth of the nodule bacteria was observed.

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PLATE 1

FIG. 1. Pieces of filter paper on plate.
FIG. 2. Effect of germinating soybean seeds on plate.

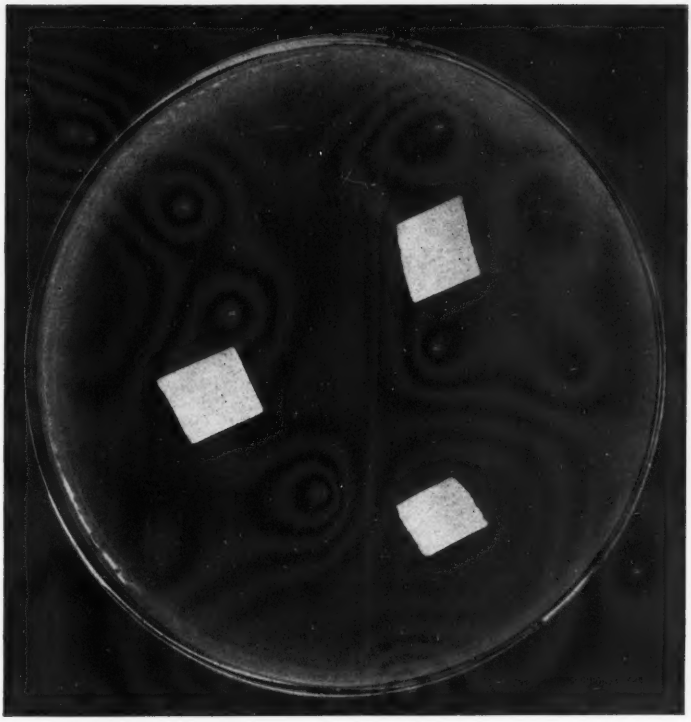

FIG. 1

FIG. 2